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(54) Title: METHOD

(57) Abstract: A method of investigating a patient's risk of developing bronchiectasis, the method comprising the step of testing the genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient. A method of selecting a course of treatment for a patient with or at risk of developing bronchiectasis the method comprising the step of testing the genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient.



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Methods

The present invention relates to methods of identifying whether a patient is likely to develop bronchiectasis, and suitable courses of treatment for such a
5 patient.

Bronchiectasis is a chronic suppurative lung disease of diverse aetiology characterised by irreversible dilatation of the bronchi and persistent purulent sputum (1). It may result from abnormal host defence
10 (hypogammaglobulinemia, ciliary dyskinesia); genetic disorders (cystic fibrosis, α -1 antitrypsin deficiency); post-infectious processes and mechanical bronchial obstruction. Several autoimmune diseases are associated with bronchiectasis. These include systemic lupus erythematosus, rheumatoid arthritis (RA) and ulcerative colitis (UC) (1).
15 Idiopathic bronchiectasis is diagnosed in those patients with chronic rhinosinusitis and bilateral lower lobe bronchiectasis in the absence of any known underlying cause.

The symptoms of bronchiectasis include a chronic cough with large
20 amounts of sputum produced, coughing up of blood, a shortness of breath, weight loss, fatigue, clubbing of fingers, wheezing, skin discolouration, paleness and breath odour. At present the standard means for diagnosing bronchiectasis includes clinical assessment, chest X-ray and high-resolution CT-thorax to determine the structure of the bronchi of the lungs.

25

Treatments of bronchiectasis include postural drainage, clapping or vibration of the patient to facilitate sputum clearance, used in conjunction with prompt treatment of respiratory infections with appropriate antibiotics. In some cases prophylactic antibiotic treatment and / or steroid inhalers and

/or selective beta₂ adrenoceptor stimulant inhalers and / or macrolides are also used.

Very little is known about the immunological processes mediating disease
5 in bronchiectasis. It has previously been shown that airway inflammation in
bronchiectasis is characterised by tissue neutrophilia, a mononuclear cell
infiltrate composed mainly of CD4⁺ T cells, CD68⁺ macrophages, and
increased interleukin-8 (IL-8) expression (2). Interleukin-8 (IL-8) is a
member of the CXC chemokine family, which acts as a potent
10 chemoattractant for neutrophils (3). IL-8 binds two specific high-affinity
soluble cell surface receptors, called CXC receptor-1 (CXCR-1) and CXC
receptor-2 (CXCR-2) (4-6). Both CXCR-1 and CXCR-2 are expressed on
neutrophils and monocytes. CXCR-1 expression is also seen on subsets of T
and NK cells (3-7). CXCR-1 binds specifically to IL-8, while CXCR-2
15 binds a variety of C-X-C cytokines (3).

Previously, single nucleotide polymorphisms have been described in the IL-
8 receptor genes (8). In the CXCR-1 gene, a polymorphism at nucleotide
+2607 (position 6334 of sequence accession number L19592) in exon 2,
20 results in a conservative amino acid substitution from serine to threonine at
the 276 amino acid residue of the CXCR-1 protein (8). Alanine scanning
mutagenesis of the receptor ligand has shown that Glu 275 and Arg 280 are
critical for ligand binding (9). The polymorphism may, therefore, have
functional significance in terms of IL-8 binding affinity to the CXCR-1
25 receptor resulting in an altered immune response in those individuals having
such a CXCR-1 genotype.

However, no differences were seen in the distribution of the polymorphism
at nucleotide +2607 of the CXCR-1 gene between control patients and

patients suffering from systemic sclerosis or cryptogenic fibrosing alveolitis (8).

A single nucleotide polymorphism in the first intron of the human
5 interferon- γ (IFN γ) gene has also been previously described. A CA repeat
microsatellite sequence in the first intron is polymorphic, allele 2 being
associated with enhanced IFN γ production (10). This allele was found to be
absolutely correlated with a T to A single nucleotide polymorphism at +874
in the IFN γ gene, a region proposed to coincide with an NF κ B binding
10 site (11). Allele 2 has been associated with the development of allograft
fibrosis in lung transplant recipients (12).

Several studies have tested the relationship between the IFN γ (+874) gene
polymorphism and IFN γ production. In healthy individuals there is a
15 relationship between high IFN γ production and the IFN γ (+874) T allele
(19). A recent study demonstrated an association between the IFN γ (+874
T/A) polymorphism and tuberculosis (18): individuals homozygous for the
IFN γ (+874) A allele had a 3.75-fold increased risk of developing
tuberculosis. Stimulated production of IFN γ from tuberculosis patients with
20 the AA genotype was about two thirds lower than in patients with other
genotypes (AT and TT) and remained depressed 6 months later compared to
control values (18). High IFN γ production *in vitro* and the presence of the T
allele has been associated with allograft fibrosis in lung transplant recipients
(12). No significant difference was found between the frequency of the
25 IFN γ polymorphism in cystic fibrosis (CF) compared to published normal
controls (17).

We have found that polymorphisms in the CXCR-1 gene and the IFN γ gene are associated with different likelihoods of developing bronchiectasis. For example, we have determined that a G/C polymorphism at nucleotide +2607 (position 6334 of sequence accession number L19592) of the CXCR-1 gene is associated with protection from and increased susceptibility to bronchiectasis in patients with ulcerative colitis. The CXCR-1 +2607 C allele is associated with increased susceptibility to bronchiectasis in patients having ulcerative colitis: individuals heterozygous for the CXCR-1 +2607 G/C polymorphism have an 11.00-fold increased risk of bronchiectasis relative to those individuals having a CXCR-1 (+2607) G/G genotype.

In addition, a polymorphism at nucleotide +874 in the IFN γ gene is also associated with protection from and increased susceptibility to bronchiectasis in patients with ulcerative colitis. Individuals with ulcerative colitis that are homozygous for the IFN γ (+874) A allele (A/A) have a 5.29-fold increased risk of bronchiectasis, while those that are homozygous for the T allele (T/T) never developed bronchiectasis in our study.

Finally, individuals with both IFN γ and CXCR-1 susceptibility genotypes A/A and G/C have a 54.67-fold increased risk of bronchiectasis.

The early diagnosis and treatment of bronchiectasis improves clinical outcome. The identification of these genetic susceptibility polymorphisms provides clinicians with markers that predict protection and susceptibility to bronchiectasis which may be used by clinicians to identify individuals who are at increased risk of developing bronchiectasis. These patients could be followed up with a view to early diagnosis to identify, and treatment to prevent progression of, bronchiectasis.

A first aspect of the invention provides for a method of investigating a patient's risk of developing bronchiectasis, the method comprising the step of testing the genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient.

5

A second aspect of the invention provides for a method of selecting a course of treatment for a patient with or at risk of developing bronchiectasis, the method comprising the step of testing the genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient.

10

The patient is a human patient with or at risk of developing bronchiectasis, for example the patient may have an existing immune dysregulation condition, such as an autoimmune disorder. As has been discussed above, patients suffering from such autoimmune conditions are at risk of developing bronchiectasis.

15

Hence, in an embodiment of the first and second aspects of the invention the patient has an immune dysregulation condition, for example ulcerative colitis.

20

By 'the genotype of genes involved in IFN γ or CXCR-1 signalling' we include the genotype of IFN γ and IFN γ receptor genes, and genes which are involved in CXCR-1-signalling. Included in this is the genotype of the genes and the associated controlling regions, for example the promoter region and any intragenic controlling region, such as those found in introns.

25

Also included are polymorphisms in genes (including the promoters and intragenic controlling regions) encoding cytokines and chemokines, as well as genes encoding receptors for such molecules, and genes which encode

transcription factors which regulate the expression of such genes. This is because polymorphisms in such genes may potentially result in altered IFN γ production or CXCR-1 signalling. Examples of cytokine and chemokine genes include the IL-12 and IL-18 cytokine genes and associated receptor genes, while transcription factors genes that may be relevant include T-Bet, C-Maf and GATA-3.

As has been discussed above, patients having the immune dysregulation condition ulcerative colitis that have the IFN γ (+874) A allele, ie genotypes A/A and A/T, have an increased risk of developing bronchiectasis. Individuals homozygous for the IFN γ (+874) A allele (A/A), ie the most susceptible genotype, have a 5.29-fold increased risk of bronchiectasis.

Hence in an embodiment of the method of the first or second aspect of the invention the gene involved in IFN γ signalling is IFN γ .

Furthermore, patients having the immune dysregulation condition ulcerative colitis that have the CXCR-1 +2607 C allele, ie genotypes G/C and C/C, have an increased risk of developing bronchiectasis. Individuals heterozygous for the CXCR-1 (+2607) G/C polymorphism have an 11.00-fold increased risk of bronchiectasis.

Hence in an embodiment of the method of the first or second aspect of the invention the gene involved in CXCR-1 signalling is CXCR-1.

Patients having the immune dysregulation condition ulcerative colitis who have both the IFN γ and CXCR-1 susceptibility genotypes (A/A & G/C) have a 54.67-fold increased risk of bronchiectasis

From this it can be seen that the IFN γ +874 A allele and the CXCR-1 +2607 C allele are both associated with increased susceptibility of a patient to developing bronchiectasis.

5 Hence, the method of the first and second aspects of the invention includes the step of testing the patient's genotype at IFN γ position +874 or testing the patient's genotype at CXCR-1 position +2607.

If the patient has an IFN γ +874 A allele then the risk is increased. If the
10 patient has a CXCR-1 C allele then the risk is also increased.

From the data outlined above, it appears that a patient's risk of developing bronchiectasis is, at least in part, determined by levels of IFN γ polypeptide and by the type of CXCR-1 polypeptide present.

15

Hence the first and second aspects of the invention also include testing the IFN γ or CXCR-1 genotype of the patient to identify if the patient has any other IFN γ or CXCR-1 genotypes which may be deleterious to the individual. For example, the first intron of the human IFN γ gene has a variable-length
20 CA repeat region. The number of CA repeats can vary from 11 to 15, with the relative levels of IFN γ polypeptide having an association with the IFN γ genotype (Pravica et al (1999) Eu J Immunogenetics 26, 1-3). Therefore, testing for the presence of an IFN γ genotype which is associated with reduced IFN γ polypeptide levels may also be used in investigating a
25 patient's risk of developing bronchiectasis.

The method of the first aspect of the invention may be used as an adjunct to known assessment or prognostic methods, such as clinical assessment, chest x-rays and high-resolution CT-Thorax analysis. The method may also be

used in conjunction with consideration of other risk factors, for example the presence of primary ciliary dyskinesia, cystic fibrosis, primary and secondary immunoglobulin deficiency, a post infectious process for example, childhood infections such as whooping cough, a past medical
5 history of tuberculosis, or mechanical bronchial obstruction.

However, an advantage of the present application is that the tests listed above only indicate whether a patient has developed bronchiectasis, whereas the method of the invention allows for a screen of individuals to
10 identify those at risk, then to subsequently closely monitor at risk individuals to prevent disease; or having identified those at risk administer to them a treatment to prevent disease or treat disease early to prevent rapid progression.

15 The numbering of the DNA sequence of the IFN γ gene and the position of the DNA polymorphism at +874 is shown in Figure 1: IFN γ has the Genbank accession number. The numbering of the DNA sequence of the CXCR-1 gene and the position of the DNA polymorphism at +874 is shown in Figure 2: CXCR-1 has the Genbank accession number L19592.

20 Genotypic analysis of the IFN γ and CXCR-1 genes (or other genes, as noted above) may be performed by any one of a large number of methods as will be well known to those skilled in the art. Examples of such methods include sequencing, RFLP, ARMS PCR, CAPS PCR, PCR and sequence-specific
25 oligonucleotide hybridisation, RT-PCR, SnapShot PCR, Ligase detection reaction, PCR and Maldi-TOF, Pyrosequencing, ligase chain reaction (eg Abbott procedure) and branched DNA (eg Chiron procedure). These and other suitable techniques will be well known to those skilled in the art.

The genotype may preferably be determined by testing a sample from the patient. Preferably, the sample contains nucleic acid, such as genomic DNA or mRNA (preferably genomic DNA).

5 The sample may conveniently be whole blood or genomic DNA extracted from whole blood, for example a 5 ml sample collected into EDTA and extracted using a commercial kit (for example as supplied by Nucleon II, Scotlabs, UK).

10 Although any sample containing nucleic acid derived from the patient is useful in the method of the first aspect of the invention, it is preferred if the sample is readily obtainable from the patient, for example blood, semen or skin cells. Most conveniently the sample is blood. Although it is preferred that the sample containing nucleic acid from the patient is, or is derived
15 directly from, a cell of the patient, a sample indirectly derived from a patient, such as a cell grown in culture, is also included within the invention. Equally, although the nucleic acid derived from the patient may have been physically within the patient, it may alternatively have been copied from nucleic acid which was physically within the patient.

20

It is preferred that the method of identifying the genotype at, for example, position +874 of IFN γ or position +2607 of CXCR-1 of a patient is based on amplification refractory mutation system (ARMS) PCR, a technique well known to those skilled in the art. The method employed in the present study
25 is described in detail in Example 1.

Primers which are suitable for use in an ARMS PCR reaction to genotype IFN γ and CXCR-1 are presented above an example of the ARMS methodology is shown in Example 1. However, suitable PCR primers for

both this method and several of the other methods outlined above will be apparent to those skilled in the art and may have the following properties:

5 It is well known that the sequence at the 5' end of the oligonucleotide need not match the target sequence to be amplified.

It is usual that the PCR primers do not contain any complementary structures with each other longer than 2 bases, especially at their 3' ends, as this feature may promote the formation of an artifactual product called "primer dimer".
10 When the 3' ends of the two primers hybridize, they form a "primed template" complex, and primer extension results in a short duplex product called "primer dimer".

Internal secondary structure should be avoided in primers. For symmetric
15 PCR, a 40-60% G+C content is often recommended for both primers, with no long stretches of any one base. The classical melting temperature calculations used in conjunction with DNA probe hybridization studies often predict that a given primer should anneal at a specific temperature or that the 72 °C extension temperature will dissociate the primer/template
20 hybrid prematurely. In practice, the hybrids are more effective in the PCR process than generally predicted by simple T_m calculations.

Optimum annealing temperatures may be determined empirically and may be higher than predicted. *Taq* DNA polymerase does have activity in the
25 37-55 °C region, so primer extension will occur during the annealing step and the hybrid will be stabilized. The concentrations of the primers are equal in conventional (symmetric) PCR and, typically, within 0.1- to 1 μ M range.

When a pair of suitable nucleic acids of the invention are used in a PCR it is possible to detect the product by gel electrophoresis and ethidium bromide staining. As an alternative to detecting the product of DNA amplification using agarose gel electrophoresis and ethidium bromide staining of the DNA, it is convenient to use a labelled oligonucleotide capable of hybridising to the amplified DNA as a probe. When the amplification is by a PCR the oligonucleotide probe hybridises to the interprimer sequence as defined by the two primers. The oligonucleotide probe is preferably between 10 and 50 nucleotides long, more preferably between 15 and 30 nucleotides long. The probe may be labelled with a radionuclide such as ^{32}P , ^{33}P and ^{35}S using standard techniques, or may be labelled with a fluorescent dye. When the oligonucleotide probe is fluorescently labelled, the amplified DNA product may be detected in solution (see for example Balaguer *et al* (1991) "Quantification of DNA sequences obtained by polymerase chain reaction using a bioluminescence adsorbent" *Anal. Biochem.* **195**, 105-110 and Dilesare *et al* (1993) "A high-sensitivity electrochemiluminescence-based detection system for automated PCR product quantitation" *BioTechniques* **15**, 152-157.

PCR products can also be detected using a probe which may have a fluorophore-quencher pair or may be attached to a solid support or may have a biotin tag or they may be detected using a combination of a capture probe and a detector probe.

Fluorophore-quencher pairs are particularly suited to quantitative measurements of PCR reactions (eg RT-PCR). Fluorescence polarisation using a suitable probe may also be used to detect PCR products.

By 'selecting a course of treatment' set out in the second aspect of the invention we include both the timing of when the patient is administered therapy and the type of therapy to be used. The method of the second aspect of the invention may also be useful when used in conjunction with the other
5 known prognostic or diagnostic methods outlined above.

Therefore, the method of the second aspect of the invention may be useful in assessing whether the patient should be administered therapy at an early stage (if the risk is high) or should be monitored for longer before
10 beginning such therapy (if the risk is low, thereby avoiding unnecessary side-effects and costs).

For example, a patient with an autoimmune disorder and having a susceptible IFN γ A/A genotype at position +874 or susceptible CXCR-1
15 heterozygous G/C genotype at position +2607 can receive prompt treatment of respiratory infections with appropriate antibiotics.

Also worthy of consideration is the fact that, in the study presented in Example 1, almost all individuals with ulcerative colitis and bronchiectasis
20 had undergone a colectomy prior to developing bronchiectasis. Hence it seems that colectomy should be avoided as a treatment option if at all possible in individuals having both the IFN γ +874 A allele and the CXCR-1 +2707 C allele. If this is not possible, then patients having a colectomy should be monitored closely for the development of bronchiectasis and any
25 treatment available administered appropriately.

The method of the second aspect of the invention may also be useful in assessing the patient in order to determine type of therapy to be used.

Patients having the susceptible IFN γ A allele at position +874, for example an A/A or A/T genotype, or susceptible CXCR-1 C allele at position +2607, for example a G/C or C/C genotype, may be treated with agents that can compensate for the IFN γ or CXCR-1 genotype.

5

As has been discussed above, the IFN γ genotype is associated with reduced IFN γ production (Pravica et al (1999) *Eu J of Immunogenetics* 26, 1-3). Hence when the IFN γ genotype at position +874 is A/A or A/T, the course of treatment for the patient is IFN γ therapy. By 'IFN γ therapy' we include
10 administering to the patient a therapeutically appropriate quantity of IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide. Such a nucleic acid sequence may be a cDNA sequence or it may be a genomic DNA sequence. In the case of the latter, the IFN γ genotype at position +874 is T. Examples of the polypeptide sequence of IFN γ and the sequence of a
15 nucleic acid encoding an IFN γ polypeptide may be found in Genbank accession number M37265 and shown in Figure 1. We also include the use of any agonists or antagonists to IFN γ . Examples of agonists or antagonists of IFN γ that may be of use in IFN γ therapy are shown in Figures 3 and 4.

20 Alternatively, when the CXCR-1 genotype at position +2607 is C/C or G/C the course of treatment for the patient may be CXCR-1 therapy. By 'CXCR-1 therapy' we include administering to the patient a therapeutically appropriate quantity of CXCR-1 polypeptide or a nucleic acid sequence encoding an CXCR-1 polypeptide. Such a nucleic acid sequence may be a
25 cDNA sequence or it may be a genomic DNA sequence. In both cases the CXCR-1 genotype at position +2607 is G. Examples of the polypeptide sequence of CXCR-1 and the sequence of a nucleic acid encoding an CXCR-1 polypeptide may be found in Genbank accession number L19592 and shown in Figure 2. We also include administering to the patient a

therapeutically appropriate quantity of a CXCR-1 agonist or antagonist or any other polypeptide or nucleic acid sequence encoding a polypeptide that alters the function of CXCR-1. Examples of antagonists that may be of use in CXCR-1 therapy are shown in Figure 5.

5

Preferably, agents used in CXCR-1 therapy act specifically to alter CXCR-1 function rather than both CXCR-1 and CXCR-2 functions.

Other treatments that may be used in treating bronchiectasis, for example as part of CXCR-1 therapy or alongside IFN γ therapy, include the use of IL-8 agonists or antagonists. Examples of such agents are shown in Figure 6.

In addition to any of the above, the IFN γ or CXCR-1 therapy may include a therapeutically appropriate quantity of an antibiotic, antifungal or recombinant protein or antibodies to elements of the immune system such as chemokines, cytokines and their receptors (for example recombinant IL-12 or IL-18, or antibodies to IL-8 or IL-18).

Agents used in CXCR-1 therapy may disrupt the expression of the CXCR-1 gene. In relation to CXCR-1 or IFN γ therapy, expression may be disrupted by a compound such as an RNAi molecule, or an antisense molecule or ribozyme directed (for example, capable of binding to a polynucleotide encoding CXCR-1 or other target polynucleotide that modulates IFN γ or CXCR-1 mediated signalling, under physiological conditions) against a polynucleotide encoding CXCR-1 (or other target polynucleotide that modulates IFN γ or CXCR-1 mediated signalling). A still further aspect of the invention provides the use of a compound (for example a nucleic acid molecule) capable of disrupting the expression of CXCR-1 (or other target polynucleotide that modulates IFN γ or CXCR-1 mediated signalling) in the

manufacture of a medicament for the treatment of bronchiectasis or ulcerative colitis.

5 Examples of a nucleic acid encoding a CXCR-1 polypeptide may be found in Genbank accession number L19592 and shown in Figure 2. This information may be used to assist the design of an RNAi molecule, or an antisense molecule or ribozyme as described above.

10 RNA duplexes may be selected using the siDESIGN centre programme at <http://www.dharmacon.com/>. When testing the duplexes, for example in cultured cells, a control RNA duplex that does not target any cellular mRNA may be employed.

15 Alternatively, expression may be disrupted using an antisense oligonucleotide, for example an antisense oligonucleotide directed against a CXCR-1 gene, or other target gene as discussed above. Antisense oligonucleotides are single-stranded nucleic acid, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA
20 duplex is formed. These nucleic acids are often termed "antisense" because they are complementary to the sense or coding strand of the gene. Recently, formation of a triple helix has proven possible where the oligonucleotide is bound to a DNA duplex. It was found that oligonucleotides could recognise sequences in the major groove of the DNA double helix. A triple helix was
25 formed thereby. This suggests that it is possible to synthesise a sequence-specific molecules which specifically bind double-stranded DNA *via* recognition of major groove hydrogen binding sites.

By binding to the target nucleic acid, the above oligonucleotides can inhibit the function of the target nucleic acid. This could, for example, be a result of blocking the transcription, processing, poly(A) addition, replication, translation, or promoting inhibitory mechanisms of the cells, such as promoting RNA degradations.

Antisense oligonucleotides are prepared in the laboratory and then introduced into cells, for example by microinjection or uptake from the cell culture medium into the cells, or they are expressed in cells after transfection with plasmids or retroviruses or other vectors carrying an antisense gene. Antisense oligonucleotides were first discovered to inhibit viral replication or expression in cell culture for Rous sarcoma virus, vesicular stomatitis virus, herpes simplex virus type 1, simian virus and influenza virus. Since then, inhibition of mRNA translation by antisense oligonucleotides has been studied extensively in cell-free systems including rabbit reticulocyte lysates and wheat germ extracts. Inhibition of viral function by antisense oligonucleotides has been demonstrated *in vitro* using oligonucleotides which were complementary to the AIDS HIV retrovirus RNA (Goodchild, J. 1988 "Inhibition of Human Immunodeficiency Virus Replication by Antisense Oligodeoxynucleotides", *Proc. Natl. Acad. Sci. (USA)* **85**(15), 5507-11). The Goodchild study showed that oligonucleotides that were most effective were complementary to the poly(A) signal; also effective were those targeted at the 5' end of the RNA, particularly the cap and 5' untranslated region, next to the primer binding site and at the primer binding site. The cap, 5' untranslated region, and poly(A) signal lie within the sequence repeated at the ends of retrovirus RNA (R region) and the oligonucleotides complementary to these may bind twice to the RNA.

Oligonucleotides are subject to being degraded or inactivated by cellular endogenous nucleases. To counter this problem, it is possible to use modified oligonucleotides, eg having altered internucleotide linkages, in which the naturally occurring phosphodiester linkages have been replaced with another linkage. For example, Agrawal *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7079-7083 showed increased inhibition in tissue culture of HIV-1 using oligonucleotide phosphoramidates and phosphorothioates. Sarin *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7448-7451 demonstrated increased inhibition of HIV-1 using oligonucleotide methylphosphonates. Agrawal *et al* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7790-7794 showed inhibition of HIV-1 replication in both early-infected and chronically infected cell cultures, using nucleotide sequence-specific oligonucleotide phosphorothioates. Leither *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3430-3434 report inhibition in tissue culture of influenza virus replication by oligonucleotide phosphorothioates.

15

Oligonucleotides having artificial linkages have been shown to be resistant to degradation *in vivo*. For example, Shaw *et al* (1991) in *Nucleic Acids Res.* **19**, 747-750, report that otherwise unmodified oligonucleotides become more resistant to nucleases *in vivo* when they are blocked at the 3' end by certain capping structures and that uncapped oligonucleotide phosphorothioates are not degraded *in vivo*.

20

A detailed description of the H-phosphonate approach to synthesising oligonucleoside phosphorothioates is provided in Agrawal and Tang (1990) *Tetrahedron Letters* **31**, 7541-7544, the teachings of which are hereby incorporated herein by reference. Syntheses of oligonucleoside methylphosphonates, phosphorodithioates, phosphoramidates, phosphate esters, bridged phosphoramidates and bridge phosphorothioates are known in the art. See, for example, Agrawal and Goodchild (1987) *Tetrahedron Letters*

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28, 3539; Nielsen *et al* (1988) *Tetrahedron Letters* **29**, 2911; Jager *et al* (1988) *Biochemistry* **27**, 7237; Uznanski *et al* (1987) *Tetrahedron Letters* **28**, 3401; Bannwarth (1988) *Helv. Chim. Acta.* **71**, 1517; Crosstick and Vyle (1989) *Tetrahedron Letters* **30**, 4693; Agrawal *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1401-1405, the teachings of which are incorporated herein by reference. Other methods for synthesis or production also are possible. In a preferred embodiment the oligonucleotide is a deoxyribonucleic acid (DNA), although ribonucleic acid (RNA) sequences may also be synthesised and applied.

10

The oligonucleotides useful in the invention preferably are designed to resist degradation by endogenous nucleolytic enzymes. *In vivo* degradation of oligonucleotides produces oligonucleotide breakdown products of reduced length. Such breakdown products are more likely to engage in non-specific hybridization and are less likely to be effective, relative to their full-length counterparts. Thus, it is desirable to use oligonucleotides that are resistant to degradation in the body and which are able to reach the targeted cells. The present oligonucleotides can be rendered more resistant to degradation *in vivo* by substituting one or more internal artificial internucleotide linkages for the native phosphodiester linkages, for example, by replacing phosphate with sulphur in the linkage. Examples of linkages that may be used include phosphorothioates, methylphosphonates, sulphone, sulphate, ketyl, phosphorodithioates, various phosphoramidates, phosphate esters, bridged phosphorothioates and bridged phosphoramidates. Such examples are illustrative, rather than limiting, since other internucleotide linkages are known in the art. See, for example, Cohen, (1990) *Trends in Biotechnology*. The synthesis of oligonucleotides having one or more of these linkages substituted for the phosphodiester internucleotide linkages is well known in

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the art, including synthetic pathways for producing oligonucleotides having mixed internucleotide linkages.

Oligonucleotides can be made resistant to extension by endogenous enzymes
5 by “capping” or incorporating similar groups on the 5' or 3' terminal nucleotides. A reagent for capping is commercially available as Amino-Link IITM from Applied BioSystems Inc, Foster City, CA. Methods for capping are described, for example, by Shaw *et al* (1991) *Nucleic Acids Res.* **19**, 747-750 and Agrawal *et al* (1991) *Proc. Natl. Acad. Sci. USA* **88(17)**, 7595-7599, the
10 teachings of which are hereby incorporated herein by reference.

A further method of making oligonucleotides resistant to nuclease attack is for them to be “self-stabilised” as described by Tang *et al* (1993) *Nucl. Acids Res.* **21**, 2729-2735 incorporated herein by reference. Self-stabilised
15 oligonucleotides have hairpin loop structures at their 3' ends, and show increased resistance to degradation by snake venom phosphodiesterase, DNA polymerase I and fetal bovine serum. The self-stabilised region of the oligonucleotide does not interfere in hybridization with complementary nucleic acids, and pharmacokinetic and stability studies in mice have shown
20 increased *in vivo* persistence of self-stabilised oligonucleotides with respect to their linear counterparts.

It will be appreciated that antisense agents also include larger molecules which bind to the CXCR-1 polypeptide mRNA or genes (or other target gene)
25 and substantially prevent expression of said CXCR-1 polypeptide (or other polypeptide) mRNA or genes and substantially prevent expression of said CXCR-1 polypeptide. Thus, expression of an antisense molecule which is substantially complementary to said CXCR1 polypeptide mRNA is envisaged as part of the invention.

The said larger molecules (or molecules encoding CXRC1 polypeptides, or other polypeptides, for example IFN γ , as indicated above) may be expressed from any suitable genetic construct as is described below and delivered to the patient. Typically, the genetic construct which expresses the antisense molecule comprises at least a portion of the said interacting polypeptide coding sequence operatively linked to a promoter which can express the antisense molecule in the cell. Suitable promoters will be known to those skilled in the art, and may include promoters for ubiquitously expressed, for example housekeeping genes or for tissue-specific genes, depending upon where it is desired to express the antisense (or other) molecule.

Although the genetic construct can be DNA or RNA it is preferred if it is DNA.

Preferably, the genetic construct is adapted for delivery to a human cell.

Means and methods of introducing a genetic construct into a cell in an animal body are known in the art. For example, the constructs of the invention may be introduced into the cells by any convenient method, for example methods involving retroviruses, so that the construct is inserted into the genome of the (dividing) cell.

Other methods involve simple delivery of the construct into the cell for expression therein either for a limited time or, following integration into the genome, for a longer time. An example of the latter approach includes liposomes (Nassander *et al* (1992) *Cancer Res.* **52**, 646-653). Other methods of delivery include adenoviruses carrying external DNA via an antibody-polylysine bridge (see Curiel *Prog. Med. Virol.* **40**, 1-18) and

transferrin-polycation conjugates as carriers (Wagner *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3410-3414). The DNA may also be delivered by adenovirus wherein it is present within the adenovirus particle. It will be appreciated that "naked DNA" and DNA complexed with cationic and neutral lipids may also be useful in introducing the DNA of the invention
5 into cells of the patient to be treated. Non-viral approaches to gene therapy are described in Ledley (1995) *Human Gene Therapy* **6**, 1129-1144. Alternative targeted delivery systems are also known such as the modified adenovirus system described in WO 94/10323 wherein, typically, the DNA
10 is carried within the adenovirus, or adenovirus-like, particle. Michael *et al* (1995) *Gene Therapy* **2**, 660-668 describes modification of adenovirus to add a cell-selective moiety into a fibre protein. Mutant adenoviruses which replicate selectively in p53-deficient human tumour cells, such as those described in Bischoff *et al* (1996) *Science* **274**, 373-376 are also useful for
15 delivering the genetic construct of the invention to a cell. Thus, it will be appreciated that a further aspect of the invention provides a virus or virus-like particle comprising a genetic construct of the invention. Other suitable viruses or virus-like particles include HSV, AAV, vaccinia and parvovirus.

20 A ribozyme capable of cleaving the CXCR-1 (or other) polypeptide RNA or DNA may also be useful for reducing expression of the CXCR-1 (or other) polypeptide. A gene expressing said ribozyme may be administered in substantially the same and using substantially the same vehicles as for the antisense molecules. Ribozymes which may be encoded in the genomes of
25 the viruses or virus-like particles herein disclosed are described in Cech and Herschlag "Site-specific cleavage of single stranded DNA" US 5,180,818; Altman *et al* "Cleavage of targeted RNA by RNase P" US 5,168,053, Cantin *et al* "Ribozyme cleavage of HIV-1 RNA" US 5,149,796; Cech *et al* "RNA ribozyme restriction endoribonucleases and methods", US 5,116,742;

Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endonucleases and methods", US 5,093,246; and Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endoribonucleases and methods; cleaves single-stranded RNA at specific site by transesterification", US 4,987,071, all incorporated herein by reference.

The genetic constructs of the invention can be prepared using methods well known in the art.

10 A third aspect of the invention is a pharmaceutical composition comprising:

a) an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide, or an agonist or antagonist of IFN γ ; and,

15 b) a CXCR-1 polypeptide or soluble portion thereof or a nucleic acid sequence encoding a CXCR-1 polypeptide or soluble portion thereof, or an agonist or antagonist of CXCR-1; and,

c) a pharmaceutically acceptable excipient.

20

The pharmaceutical composition of this aspect of the invention may be of use in treating patients with or at risk of developing bronchiectasis. For example a patient who has been determined using the method of the first aspect of the invention to be at risk of developing bronchiectasis may be treated using the pharmaceutical composition presented in this aspect of the invention. Alternatively, the pharmaceutical composition may be of use in the method of treatment selected for a patient using the method of the second aspect of the invention.

The pharmaceutical composition may comprise an antagonist or agonist of CXCR-1. Examples of suitable agonists or antagonists of CXCR-1 are discussed above. This includes the use of antisense and RNAi molecules to alter CXCR-1 expression, as outlined above.

5

In addition, the pharmaceutical composition may further comprise an agonist or antagonist of IFN γ . Examples of suitable agonists or antagonists of IFN γ are discussed above. The pharmaceutical composition may further comprise an IL-8 agonist or antagonist. Examples of such agents are shown
10 in Figure 6 and are discussed above. The pharmaceutical composition may further comprise an antibiotic or an antifungal agent or a recombinant protein or antibodies to elements of the immune system, for example chemokines and cytokines.

15 Finally, the pharmaceutical composition may further comprise a therapeutically appropriate quantity of a recombinant protein or antibodies to elements of the immune system such as chemokines, cytokines and their receptors (for example recombinant IL-12 or IL-18, or antibodies to IL-8 or IL-18).

20

A further embodiment of this aspect of the invention is wherein the pharmaceutical composition further comprises a therapeutically appropriate quantity of steroids or agents that suppress the immune response. Examples of such steroids or agents include immunosuppressants used to treat
25 autoimmune disease and used in transplantation to prevent rejection of the graft.

Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

The compounds of this aspect of the invention may be administered orally, intranasally, inhalation or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in
5 the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

10 In human therapy, the compounds of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

15 For example, the compounds of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The compounds of invention may also be administered *via* intracavernosal
20 injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca
25 starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as

magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in
5 gelatin capsules. Preferred excipients in this regard include lactose, starch,
a cellulose, milk sugar or high molecular weight polyethylene glycols. For
aqueous suspensions and/or elixirs, the compounds of the invention may be
combined with various sweetening or flavouring agents, colouring matter or
dyes, with emulsifying and/or suspending agents and with diluents such as
10 water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for
example, intravenously, intra-arterially, intraperitoneally, intrathecally,
intraventricularly, intrasternally, intracranially, intra-muscularly or
15 subcutaneously, or they may be administered by infusion techniques. They
are best used in the form of a sterile aqueous solution which may contain
other substances, for example, enough salts or glucose to make the solution
isotonic with blood. The aqueous solutions should be suitably buffered
(preferably to a pH of from 3 to 9), if necessary. The preparation of suitable
20 parenteral formulations under sterile conditions is readily accomplished by
standard pharmaceutical techniques well-known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-
aqueous sterile injection solutions which may contain anti-oxidants, buffers,
25 bacteriostats and solutes which render the formulation isotonic with the blood
of the intended recipient; and aqueous and non-aqueous sterile suspensions
which may include suspending agents and thickening agents. The
formulations may be presented in unit-dose or multi-dose containers, for
example sealed ampoules and vials, and may be stored in a freeze-dried

(lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

5

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention will usually be from 1 to 1000 mg per adult (*i.e.* from about 0.015 to 15 mg/kg), administered in single or divided doses.

10

Thus, for example, the tablets or capsules of the compound of the invention may contain from 1 mg to 1000 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention.

15

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

20

The compounds of the invention can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, *e.g.*

25

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be
5 determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, *e.g.* using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.* sorbitan trioleate. Capsules and cartridges (made, for example, from
10 gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each
15 metered dose or "puff" contains at least 1 mg of a compound of the invention for delivery to the patient. It will be appreciated that the overall daily dose with an aerosol will vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

20

Further methods of administering the compounds of the invention include nasal sprays or nasal drops as would be appreciated by a person skilled in the art.

25 Generally, in humans, oral, intranasal, inhalation or topical administration of the compounds of the invention is the preferred route, being the most convenient. In circumstances where the recipient suffers from a swallowing disorder or from impairment of drug absorption after oral

administration, the drug may be administered parenterally, *e.g.* sublingually or buccally.

A fourth aspect of the invention is the use of an IFN γ polypeptide or a
5 nucleic acid sequence encoding a IFN γ polypeptide or an IFN γ agonist or
antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence
encoding a CXCR-1 polypeptide or a CXCR1 agonist or antagonist and/or a
pharmaceutical composition according to the third aspect of the invention in
the manufacture of a medicament for the treatment of a patient with or at
10 risk of developing bronchiectas.

The pharmaceutical composition may be suitable for presentation to the
patient as a pharmaceutical formulation as described above.

15 A fifth aspect of the invention is a method of treating a patient with or at
risk of developing bronchiectasis by administering a therapeutically
appropriate quantity of an IFN γ polypeptide or a nucleic acid sequence
encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a
CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1
20 polypeptide or a CXCR1 agonist or antagonist and/or a pharmaceutical
composition according to the third aspect of the invention.

A patient with or at risk of developing bronchiectas may be identified using
the first aspect of the invention. The medicament may be prescribed to a
25 patient as a result of the selection process of the second aspect of the
invention.

The patient is a human patient with or at risk of developing bronchiectasis,
for example the patient may have an existing immune dysregulation

condition, such as an autoimmune disorder. As has been discussed above, patients suffering from such autoimmune conditions are at risk of developing bronchiectasis. Hence a suitable patient would include a patient having an immune dysregulation condition, for example ulcerative colitis.

5

A sixth aspect of the invention is the use of an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR1 agonist or antagonist and/or a
10 pharmaceutical composition according to the third aspect of the invention in the manufacture of a medicament for the treatment of a patient with or at risk of developing ulcerative colitis.

The pharmaceutical composition may be suitable for presentation to the
15 patient as a pharmaceutical formulation as described above.

A seventh aspect of the invention is a method of treating a patient with or at risk of developing ulcerative colitis by administering a therapeutically appropriate quantity of an IFN γ polypeptide or a nucleic acid sequence
20 encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR1 agonist or antagonist and/or a pharmaceutical composition according to the third aspect of the invention.

25 Patients with or at risk of developing ulcerative colitis may benefit from the treatments of the invention outlined above.

Suitable IFN γ polypeptides, nucleic acid sequences encoding IFN γ polypeptides, CXCR-1 polypeptides and nucleic acid sequences encoding

CXCR-1 polypeptides for use in the fourth, fifth, sixth or seventh aspects of the invention are discussed above in relation to the second aspect of the invention.

- 5 Suitable agonists or antagonists of IFN γ or CXCR-1 are discussed above in relation to the second aspect of the invention.

A eighth aspect of the invention is a kit of parts comprising:

- 10 a) an IFN γ polypeptide or a nucleic acid sequence encoding a IFN γ polypeptide or an IFN γ agonist or antagonist and/or CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to the third aspect of the
15 invention; and,

- b) polynucleotides suitable for use in the method of the first or second aspects of the invention.

- 20 Suitable polynucleotides that can be used in the method of the first or second aspect of the invention to determine the IFN γ or CXCR-1 genotype of a patient are set out in the accompanying example.

- A ninth aspect of the invention is a kit of parts comprising polynucleotides
25 for testing the IFN γ genotype and for testing the CXCR-1 genotype.

Polynucleotides which may be included in this aspect of the invention are displayed in the accompanying example.

All documents referred to herein are hereby incorporated by reference.

The invention is now described in more detail by reference to the following, non-limiting Examples and Figures.

5

Figure 1: Sequence listing of IFN γ

A. Polynucleotide sequence of IFN γ gene. The polymorphic nucleotide at position +875 is shown in bold.

B. Polypeptide sequence of IFN γ .

10

Figure 2: Sequence listing of CXCR-1

A. Polynucleotide sequence of CXCR-1 gene. The polymorphic nucleotide at position +2607 is shown in bold.

B. Polypeptide sequence of CXCR-1.

15

Figure 3: Agonists of IFN γ

Figure 4: Antagonists of IFN γ

20 **Figure 5: Antagonists of CXCR-1.**

Figure 6: Agonists and antagonists of IL-8

25 **Example 1: Interferon- γ and CXC Receptor 1 Gene Polymorphisms in Bronchiectasis associated with Ulcerative Colitis.**

Summary

Background Bronchiectasis is a chronic lung disease that is sometimes associated with rheumatoid arthritis and ulcerative colitis. This study investigates single base change polymorphisms identified in the first intron of interferon γ (+874 T/A) and the second exon of the CXCR1 gene (+2607 G/C) and bronchiectasis susceptibility. Interferon γ (+874) A homozygosity is associated with reduced interferon γ production, while CXCR1 binds interleukin-8, leading to chemoattraction of neutrophils.

Methods A UK population of 98 patients with idiopathic bronchiectasis; 12 with rheumatoid arthritis and bronchiectasis; 10 with ulcerative colitis and bronchiectasis, and control groups were studied. DNA samples were genotyped for IFN- γ and CXCR1 polymorphisms by polymerase chain reaction.

Findings Individuals homozygous for the interferon- γ (+874) A allele had 5.29-fold increased risk of bronchiectasis associated with ulcerative colitis (95% confidence interval, 1.27–22.10, $p < 0.02$), while homozygosity for the T allele was never observed in this group ($p < 0.0001$). Individuals heterozygous for the CXCR1 (+2607) G/C polymorphism had 11.00-fold increased risk of bronchiectasis associated with ulcerative colitis (95% confidence interval, 2.55–47.42, $p < 0.003$). Individuals with both the interferon- γ and CXCR1 susceptibility genotypes (A/A & G/C) had 54.67-fold increased risk of bronchiectasis associated with ulcerative colitis (95% confidence interval, 5.25–569.15, $p < 0.0003$).

Interpretation The identification of these genetic susceptibility polymorphisms suggests that immune dysregulation is involved in development of bronchiectasis in individuals with ulcerative colitis. It also

provides clinicians with markers that predict increased susceptibility to this rare, but important complication of ulcerative colitis enabling early diagnosis and treatment.

5 Introduction

Bronchiectasis is a chronic suppurative lung disease of diverse aetiology characterized by irreversible dilatation of the bronchi and persistent purulent sputum (1). It may result from abnormal host defence (hypogammaglobulinemia, ciliary dyskinesia); genetic disorders (cystic
10 fibrosis, α -1 antitrypsin deficiency); post-infectious processes and mechanical bronchial obstruction. Several autoimmune diseases are associated with bronchiectasis. These include systemic lupus erythematosus, rheumatoid arthritis (RA) and ulcerative colitis (UC) (1). Idiopathic bronchiectasis is diagnosed in those patients with chronic
15 rhinosinusitis and bilateral lower lobe bronchiectasis in the absence of any known underlying cause. Very little is known about the immunological processes mediating disease in bronchiectasis. It has been shown that airway inflammation in bronchiectasis is characterised by tissue neutrophilia, a mononuclear cell infiltrate composed mainly of $CD4^+$ T
20 cells, $CD68^+$ macrophages, and increased interleukin-8 (IL-8) expression (2). Interleukin-8 (IL-8) is a member of the CXC chemokine family, which acts as a potent chemoattractant for neutrophils (3). IL-8 binds two specific high-affinity soluble cell surface receptors, called CXC receptor-1 (CXCR-1) and CXC receptor-2 (CXCR-2) (4-6). Both CXCR-1 and CXCR-2 are
25 expressed on neutrophils and monocytes. CXCR-1 expression is also seen on subsets of T and NK cells (3-7). CXCR-1 binds specifically to IL-8, while CXCR-2 binds a variety of C-X-C cytokines (3). Single nucleotide polymorphisms have been described in IL-8 and the IL-8 receptor genes (8). In the CXCR-1 gene, a polymorphism at nucleotide +2607 (position 6334 of

sequence accession number L19592) in exon 2, results in a conservative amino acid substitution from serine to threonine at the 276 amino acid residue of the CXCR-1 protein (8). Alanine scanning mutagenesis of the receptor ligand has shown that Glu 275 and Arg 280 are critical for ligand binding (9)

A single nucleotide polymorphism in the first intron of the human interferon- γ (IFN- γ) gene has been described. A CA repeat microsatellite sequence in the first intron is polymorphic, allele 2 being associated with enhanced IFN γ production (10). This allele is absolutely correlated with a T to A single nucleotide polymorphism at +874 in the IFN- γ gene, a region proposed to coincide with an NF- κ B binding site (11). Allele 2 has been associated with the development of allograft fibrosis in lung transplant recipients (12).

The aim of this study was to test the hypothesis that genetic polymorphisms in the IFN- γ or CXCR-1 gene are associated with susceptibility to idiopathic bronchiectasis, or bronchiectasis associated with RA or UC.

20 Patients and methods

Patients

Venous blood samples were collected from individuals attending the outpatient clinic of Royal Brompton & Harefield NHS Trust and St Mary's Hospital NHS Trust, London, UK. The study groups consisted of 98 patients with idiopathic bronchiectasis; 12 with rheumatoid arthritis and bronchiectasis; and 10 with ulcerative colitis and bronchiectasis. Bronchiectasis was defined structurally as damage to the bronchial wall resulting in irreversible dilatation of the bronchi, whatever the cause. Bronchiectasis was diagnosed clinically and confirmed by high resolution

computed tomographic (CT) scanning of the thorax. Idiopathic bronchiectasis was diagnosed in patients with chronic rhinosinusitis and bilateral lower lobe bronchiectasis in the absence of any underlying cause. Individuals with a diagnosis of bronchiectasis in addition to an established
5 diagnosis of either RA (defined clinically and radiologically) or UC (confirmed by biopsy) were also recruited.

Two control groups were selected from patients attending outpatients with other respiratory diseases. The first control group consisted of 85 patients
10 with chronic obstructive pulmonary disease (COPD). COPD was diagnosed according to British Thoracic Society guidelines (13). The second control group consisted of 14 patients with primary ciliary dyskinesia (PCD) and bronchiectasis. Patients with PCD have a similar pathological end point, but resulting from a genetically determined structural defect. PCD is
15 characterised by defective motility of cilia resulting in impaired mucociliary clearance and infertility (14). PCD was diagnosed clinically and confirmed functionally and morphologically (saccharine test > 60 minutes and abnormal ciliary beat frequency/dyskinetic beat pattern) at the Brompton PCD clinic. A third group of control individuals with a diagnosis
20 of UC based on clinical, radiological and histological criteria was also studied. DNA was used from these patients, recruited in Oxford with ethical approval from the Oxford Regional Ethics Committee. This study was approved by the Brompton Harefield & NHLI Ethics Committee and the St Mary's Local Research Ethics Committee. All participants gave written,
25 informed consent.

Methods

Genomic DNA was extracted from peripheral blood using a high-salt technique as previously described (15). Individual samples were genotyped

for IFN- γ (position +874) (11) and CXCR-1 (position +2607) (8) by amplification refractory mutation system polymerase chain reaction (ARMS-PCR) using primers as previously described (Table 1). Briefly, genomic DNA was amplified using *Taq* polymerase (Bioline, UK) in two
5 separate PCRs for each polymorphism; each reaction employed a generic antisense primer and one of the two allele specific sense primers. For IFN- γ genotyping an internal control product of 426 bp was amplified using primers designed from the nucleotide sequence of human growth hormone (11). For the CXCR-1 product, a 796 bp HLA-DRB internal control was
10 used, as previously described (8). After an initial denaturation step (5min, 95°C) samples were subjected to 30 rounds of 95°C for 30s, 56°C for 30s and 72°C for 1 min, with a final extension time of 5 min at 72°C. PCR products were separated by polyacrylamide gel electrophoresis and visualized by ethidium bromide staining.

Table 1. Characteristics of CXCR-1 and interferon- γ polymorphisms and primer sequences used for amplification refractory mutation system-polymerase chain reaction method

Polymorphism, Location allele	Product size (bp)	Sequence
CXCR-1 (+2607 exon 2)	Generic primer (antisense)	tcagagggttggaagagacatt
CXCR-1*G	205	Primer G (sense) cccaggatgccaggagag
CXCR-1*C	205	Primer C (sense) cccaggatgccaggagac
Internal Control	796	
DRB (exon 3)		Primer DRB3 (sense) tgccaagtggagcaccctaa
DRB (exon 4)		Primer DRB4 (antisense) gcattctgtctgtgcagat
IFN- γ (+874 intron 1)	Generic primer (antisense)	tcaacaaagctgatactcca
IFN- γ *A	261	Primer A (sense) ttcttacaacacacaaaatcaaatca
IFN- γ *T	261	Primer T (sense) ttcttacaacacacaaaatcaaatct
Internal control	426	Primer 1 (sense) gccttcccaaccattccctta
		Primer 2 (antisense) tcaggatttctgtgtgttc

Definition of abbreviations: IFN- γ = interferon- γ ; CXCR-1 = CXCR-1 = CXC receptor 1. See Reference 8, 11.

Statistical Analysis

Statistical analysis was carried out according to published guidelines (16). Differences in allele and genotype frequencies were determined by direct counting and examined for statistical significance by the chi-square test for independence, and with the Fisher exact test when appropriate. Differences in
5 disease susceptibility were expressed as odds ratios (OR) and 95% confidence intervals (95% CI). $p < 0.05$ was considered significant.

Results

10 A total of 219 people, of whom 104 (47%) were male with a median age of 63 years (mean $\pm$ SEM = 60.3 ± 1.0), were studied.

The relative frequencies of polymorphisms IFN- γ (+874) and CXCR-1 (+2607) are shown in Tables 2 and 3 respectively and are compared with control groups
15 with COPD, PCD and UC as well as published frequencies of these polymorphisms in normal populations (8,17). There were no significant differences between the frequencies of these alleles and genotypes in individuals with idiopathic bronchiectasis, or bronchiectasis associated with RA and the COPD control subjects; (since the genotyping of the COPD control group did not
20 differ significantly from either the PCD controls, UC controls or published control values, statistical values are given for the comparison between results obtained in this study for patients with bronchiectasis compared to COPD controls). Neither idiopathic bronchiectasis nor bronchiectasis associated with RA was associated with the IFN- γ (+874 T/A) polymorphism (Table 2). However, the IFN- γ +874 A
25 allele was significantly associated with increased susceptibility to bronchiectasis in association with UC. Individuals with two A alleles were markedly overrepresented among individuals with bronchiectasis associated with ulcerative colitis as compared with COPD control subjects (OR, 5.29; 95% CI, 1.27-22.10) and normal population control subjects (OR, 8.92; 95% CI, 2.19-36.33).
30 Furthermore, a T homozygous genotype was never seen in individuals with

bronchiectasis and UC ($p < 0.0001$). It has been previously shown that allele A homozygous individuals produce significantly lower levels of IFN- γ compared with individuals carrying one or two copies of allele T (18,19). The T to A polymorphism is at the end of the CA repeat region in the first intron of the human IFN- γ gene (+874*T/A). The presence of the T allele has been correlated with the presence of an enhanced IFN- γ producing microsatellite allele associated with lung allograft fibrosis (10-12).

We then investigated the relationship between CXCR-1 polymorphisms and disease in these study groups (Table 3). Again, no statistically significant differences were observed between idiopathic bronchiectatics, or bronchiectatics associated with RA and controls for the CXCR-1 (+2607) G/C polymorphism (Table 3). However, the CXCR-1 +2607 C allele was associated with increased susceptibility to bronchiectasis in association with UC. Individuals with a heterozygous genotype for the CXCR-1 (+2607) G/C allele had an 11.00-fold increased risk of bronchiectasis associated with UC (95% CI, 2.55 – 47.42, $p < 0.003$).

Table 4 shows the genotype frequencies of individuals with bronchiectasis associated with UC compared to controls for both CXCR1 (+2607) and IFN- γ (+874) polymorphisms. Individuals with both susceptibility genotypes (A/A & G/C) have a 54.67-fold increased risk of bronchiectasis associated with UC (95% CI, 5.25 – 569.15, $p < 0.0003$) indicating that in the presence of both susceptibility genotypes the risk is multiplied rather than additive.

Table 2. Interferon- γ allele and genotype frequencies in with individuals with bronchiectasis and control subjects.

Allele/genotype frequency, number (%)											
Gene	Allele	Controls (COPD) n = 170	Controls (PCD & Bronchiectas) n = 28	OR	Control s (UC) n=89	OR	Idiopathic Bronchiectasis n = 196	OR	RA Bronchiectasis n = 24	& Bronchiectasis UC n = 20	Normal Population (%) n = 164 (17)
IFN γ +874	A	95 (56)	16 (57)	1.02	106 (60)	1.10	97 (49)	0.77	12 (50)	17 (85)	1.52*
	T	75 (44)	12 (43)	0.95	72 (40)	0.86	99 (51)	1.29	12 (50)	3 (15)	0.22*
	AA	26 (30)	4 (29)	0.91	30 (34)	1.15	25 (26)	0.77	4 (33)	7 (70)	5.29***
	AT	43 (51)	8 (57)	1.30	46 (52)	1.04	47 (48)	0.90	4 (33)	3 (30)	0.42
	TT	16 (19)	2 (14)	0.72	13(15)	0.74	26 (26)	1.56	4 (33)	0 (0)	0.00***

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; RA = rheumatoid arthritis; PCD = primary ciliary dyskinesia; UC = ulcerative colitis; IFN- γ = interferon- γ . Odds ratios (OR) and p values are compared to control COPD individuals. Statistical analyses of the allele and genotype frequencies were done by chi-square test or Fisher exact test where appropriate. Comparison between individuals with bronchiectasis & UC and controls (COPD) *P < 0.01; **P < 0.02; ***P < 0.0001. See Reference 17.

Table 3. CXCR receptor-1 allele and genotype frequencies in with individuals with bronchiectasis and control subjects.

Gene	Allele	Control (COPD) n = 170	Control (PCD & Bronchiectasis) n = 28	Control (UC) n = 89	Idiopathic Bronchiectasis n = 196	RA & Bronchiectasis n = 24	UC & Bronchiectasis n = 20	Normal population n = 388 (8)
CXCR	G	161 (95)	27 (96)	151	184 (94)	23 (96)	15 (75)	358(92)
-1	C	9 (5)	1 (4)	6 (3)	12 (6)	1 (4)	5 (25)	30(8)
+2607	GG	77 (90)	13 (93)	83 (93)	86 (88)	11 (92)	5 (50)	ND
	GC	7 (8)	1 (7)	6 (7)	12 (12)	1 (8)	5 (50)	ND
	CC	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	ND

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; RA = rheumatoid arthritis; PCD = primary ciliary dyskinesia; UC = ulcerative colitis; CXCR-1 = CXCR receptor 1. Odds ratios (OR) and p values are compared to control COPD individuals. Statistical analysis of the allele and genotype frequencies were done by chi-square test or Fisher exact test where appropriate. Comparison between individuals with bronchiectasis & UC and controls (COPD) *p < 0.003. See Reference 8.

Table 4. CXC receptor-1 and interferon- γ genotype frequencies in with individuals with bronchiectasis associated with ulcerative colitis and control subjects.

Genotype	Controls	UC	& odds
IFN- γ (+874) / CXCRC-1 (COPD)		Bronchiectasi	ratio
(+2607)		s	(OR)
	n = 83		
		n = 10	
TT / GG	15	0	0.00
TT / GC	1	0	0.00
AT / GG	37	2	0.39
AT / GC	4	1	2.19
AA / GG	24	3	1.05
AA / GC	1	4	54.67*

5

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; UC = ulcerative colitis; CXCRC-1 = CXC receptor 1. IFN- γ = interferon- γ . Odds ratios (OR) and p values are compared to control COPD individuals. Statistical analysis of the genotype frequencies was done by Fisher exact test. Comparison between individuals with bronchiectasis & UC and controls (COPD) *p < 0.00034.

10

15

Discussion

The pathogenesis underlying the inflammatory events in bronchiectasis is poorly understood and the occurrence of this disease in association with autoimmune diseases such as UC raises particularly challenging questions.

5 The existence of a subgroup of individuals with bronchiectasis in association with autoimmune disease is presumed to indicate a common denomination in immune dysregulation. This study reports novel findings which shed some light on events and may help to define disease mechanisms. We demonstrate that the IFN- γ (+874 T/A) and CXCR-1

10 (+2607 G/C) polymorphisms are significantly associated with susceptibility to bronchiectasis associated with UC. Individuals homozygous for the IFN- γ +874 A allele show a 5.29-fold increased risk of the disease, while homozygosity for the T allele is not seen in bronchiectasis and UC. Individuals with a heterozygous genotype for CXCR-1 +2607 G/C allele

15 have an 11.00-fold increased risk of bronchiectasis associated with UC. Furthermore, in the presence of both susceptibility genotypes (A/A & G/C) there is a 54.67-fold increased risk of bronchiectasis associated with UC. Neither idiopathic bronchiectasis nor bronchiectasis associated with RA are associated with either the IFN- γ (+874 T/A) or the CXCR-1 (+2607 G/C)

20 polymorphism.

Bronchiectasis is thought of as a structural endpoint that can be reached by several pathological routes ranging from mechanical obstruction (foreign body) to postinfectious damage (*Mycobacterium tuberculosis*), genetic

25 defects (CF), abnormal host defence (ciliary dyskinesia & hypogammaglobulinemia) and autoimmune disease (SLE, RA & UC)(1). An underlying cause is found in < 40% of patients (20). Idiopathic bronchiectasis is diagnosed in patients with chronic rhinosinusitis and bilateral lower lobe bronchiectasis in the absence of any underlying cause.

Bronchiectasis is a rare complication of both RA and UC. The incidence of bronchiectasis in RA has been documented as between 3.1% and 5.2% based on clinical criteria (1). The incidence of ulcerative colitis in a recent UK population study was 13.9/10⁵ per year (21). This compares with
5 published figures from the Danish national registry that showed an annual incidence of 14.1/10⁵ and 12.6/10⁵ in women and men respectively (22,23). Lung involvement in UC has been reported to be as low as 3 of 1,400 patients (0.21%) (1). In our specialist clinic taking referrals from all over the UK, we were able to recruit 10 patients over an 18 month period. Very
10 little is known about the lung immunopathology underlying bronchiectasis. One study demonstrated tissue neutrophilia, a CD4⁺ T cell and macrophage infiltrate and increased IL-8 expression (2). Another study recently demonstrated an active neutrophil inflammation in bronchiectasis with increased levels of TNF- α , IL-8 and IL-6. The degree of inflammation was
15 exaggerated by the presence of microorganisms with potential pathogenicity (24). CD4⁺ T cells can be divided into IL-12/IL-18 driven Th1 cells that produce IFN- γ and IL-4-driven, IL-4 producing Th2 cells (25). Th1 or Th2 polarized responses have been implicated in the pathogenesis of several lung diseases. For example, atopic asthma is regarded as a Th2 driven
20 response, characterised by raised IgE, eosinophilia and local IL-4 production (26), while sarcoidosis is associated with Th1-driven responses and neutrophil infiltration (27,28). The role of Th1/Th2 polarization in bronchiectasis has not yet been studied in any detail. A possible role for NK cells is suggested by the finding that bronchiectasis is a part of a syndrome
25 seen in patients who due to a TAP-2 mutation have impaired HLA class I expression (29). Our study has addressed the issue of the role of IFN- γ and CXCR-1 gene polymorphisms in these patient groups to shed some light on possible underlying immune mechanisms in this disease.

Several studies have tested the relationship between the IFN- γ (+874) gene polymorphism and IFN- γ production. In healthy individuals there is a relationship between high IFN- γ production and the IFN- γ (+874) T allele (19). A recent study demonstrated an association between the IFN- γ (+874 T/A) polymorphism and tuberculosis (18). Individuals homozygous for the IFN- γ (+874) A allele had a 3.75-fold increased risk of developing tuberculosis. Stimulated production of IFN- γ from tuberculosis patients with the AA genotype was about two thirds lower than in patients with other genotypes (AT and TT) and remained depressed 6 months later compared to control values (18). High IFN- γ production *in vitro* and the presence of the T allele has been associated with allograft fibrosis in lung transplant recipients (12). No significant difference was found between the frequency of this polymorphism in cystic fibrosis (CF) compared to published normal controls (17). The significant findings in the present study clearly bear on aspects of immune dysregulation which in a rare subgroup of patients leads to bronchiectasis and UC. Based on studies of UC like disease in knockout mouse strains, one current view is that UC is likely to result from an abnormal response to gut bacterial flora resulting from immune dysregulation (30-32). UC has also been attributed to a Th2-cell-type induced mucosal inflammation characterized by an acute inflammatory cell exudate and/or the presence of mucosal odema (32,33). The low IFN- γ producing genotype may, therefore, increase susceptibility to bronchiectasis associated with UC with both disease sites (lung and gut) involving an inappropriate immune response to chronic exposure to common bacterial pathogens.

The CXCR-1 (+2607 G/C) gene polymorphism is of particular interest since it results in a conservative amino acid substitution from serine to threonine at the 276 amino acid residue of the CXCR-1 protein, and alanine scanning

mutagenesis of the receptor ligand has shown that Glu 275 is critical for ligand binding (3,8,9). It may, therefore, have functional significance in terms of IL-8 binding affinity to the CXCR-1 receptor resulting in an altered immune response. Further evidence to support the hypothesis that CXCR-1
5 may have a role in the pathogenesis of bronchiectasis in association with UC is that CXCR-1 receptors are strongly upregulated in the mucosal epithelium of individuals with UC and there are increased numbers of CXCR-1 positive inflammatory cells (34). The disease-associated CXCR-1 polymorphism may be involved in the dysregulated neutrophil recruitment
10 reported in bronchiectasis (24).

Our findings of genetic susceptibility to bronchiectasis and UC determined by CXCR-1 and IFN- γ polymorphisms suggest functional studies that may illuminate the nature of immune dysregulation in this rare group of patients
15 that has bearing on immune mechanisms in bronchiectasis in general. The gene polymorphisms could be used clinically as markers of increased disease susceptibility allowing clinicians to target individuals with UC at increased risk of developing bronchiectasis and enabling early diagnosis and treatment to attempt to prevent disease progression.

20

References

1. Cohen M, Sahn SA. Bronchiectasis in systemic disease. *Chest* 1999;116:1063-1074.
- 25 2. Gaga M, Bentley AM, Humbert M, Barkans J, O'Brien F, Wathen CG, Kay AB, Durham SR. Increases in CD4⁺ T lymphocytes, macrophages, neutrophils and interleukin 8 positive cells in the airways of patients with bronchiectasis. *Thorax* 1998;53:685-691.

3. Hourk R. Chemokine receptors. *Cytokine Growth Factor Rev.* 2001;12:313-35.
4. Holmes WE, Lee J, Kuang WJ, Rice GC, Wood, WI. Structure and functional expression of a human interleukin-8 receptor. *Science* 1991;253:1278-1280.
- 5 1991;253;1278-1280.
5. Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* 1991;253;1280-1283.
6. Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI. Characterization of two high affinity human interleukin-8 receptors. *J Biol Chem* 1992;267:16283-16287.
- 10 1992;267:16283-16287.
7. Morohashi H, Miyawaki T, Nomura H. Expression of both types of human interleukin-8 receptors on mature neutrophils, monocytes, and natural killer cells. *J Leukoc Biol* 1995;57:180-187.
8. Renzoni E, Lympay P, Sestini P, Pantelidis P, Wells A, Black C, Welsh K, Bunn C, Knight C, Foley P, Du Bois RM. 2000. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. *Arthritis & Rheumatism* 2000;43:1633-1640.
- 15 2000;43:1633-1640.
9. Hébert CA, Chuntharapai A, Smith M, Colby T, Kim J, Horuk R. Partial functional mapping of the human interleukin-8 type A receptor. Identification of a major ligand binding domain. *J Biol Chem* 1993;268:18549-18553.
- 20 1993;268:18549-18553.
10. Pravica V, Asderakis A, Perry C, Hajeer A, Sinnott PJ, Hutchinson IV. In vitro production of IFN- γ correlates with CA repeat polymorphism in the human IFN- γ gene. *Eur J Immunogenetics* 1999;26:1-3.
- 25 1999;26:1-3.
11. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN- γ gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN- γ production. *Human Immunol* 2000;61:863-866.

12. Awad M, Pravica V, Perrey C, Gamel AE, Yonan N, Sinnott PJ, Hutchinson IV. CA repeat allele polymorphism in the first intron of human interferon- γ gene is associated with lung allograft fibrosis. *Human Immunol* 1999;60:343-346.
- 5 13. British Thoracic Society. BTS guidelines for the management of chronic obstructive pulmonary disease. *Thorax* 1997;52(Suppl 5):S4-S6.
14. Horvath I, Loukides S, Wodehouse T, Csiszer E, Cole PJ, Kharitonov SA, Barnes PJ. Comparison of exhaled and nasal nitric oxide and exhaled carbon monoxide levels in bronchiectatic patients with and without primary ciliary dyskinesia. *Thorax* 2003;58:68-72.
- 10 15. Miller SA, SS Dykes, and HF Polesky. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl Acids Res* 1988;16:1215.
16. Dorak TM. Statistical analysis in HLA and disease association studies. Biostatistics. 2003 June. Available from: <http://home.att.net/~dorak/hla/stat.html>.
- 15 17. Arkwright PD, Pravica V, Geraghty PJ, Super M, Webb AK, Schwarz M, Hutchinson IV. End-organ dysfunction in cystic fibrosis: association with angiotensin I converting enzyme and cytokine gene polymorphism. *Am J Respir Crit Care Med* 2003;167:384-389.
- 20 18. Lopez-Maderuelo D, Arnalich F, Serantes R, Gonzalez A, Codoceo R, Madero R, Vazquez JJ, Montiel. Interferon- γ and interleukin-10 gene polymorphisms in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2003;167:970-975.
- 25 19. Warle MC, Farhan A, Metselaar HJ, Hop WC, Perrey C, Zondervan PE, Kap M, Kwekkeboom J, Ijzermans JN, Tilanus HW, Pravica V, Hutchinson IV, Bouma GJ. Are cytokine gene polymorphisms related to in vitro cytokine production profiles? *Liver Transpl* 2003 Feb;9:170-181.

20. Cole P, Flower CDR, Lavender JP. Clinical and imaging aspects of bronchiectasis. In: Potchen EJ, Grainger RG, Greene R, eds. Pulmonary radiology: The Fleischner Society. Philadelphia, PA: WB Saunders; 1993, p.242-58.
- 5 21. Rubin GP, Hungin APS, Kelly PJ, Ling J. Inflammatory bowel disease: epidemiology and management in an English general practice population. *Aliment Pharmacol Ther* 2000;14:1553-1559.
22. Fonager K, Sorensen HT, Oslen J. Change in incidence of Crohn's disease and ulcerative colitis in Denmark. A study based on the National
10 Registry of Patients, 1981-1992. *Int J Epidemiol* 1997;26:1003-1008.
23. Logan RFA. Inflammatory bowel disease incidence: up, down or unchanged? *Gut* 1998;42:309-311.
24. Angrill J, Agusti C, Celis RD, Filella X, Rano A, Elena M, Bellacasa JP, Xaubet A, Torres A. Bronchial inflammation and colonization in
15 patients with clinically stable bronchiectasis. *Am J Resp Crit Care Med* 2001;164:1628-1632.
25. Mosmann TR, Coffman, RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. 1989. *Ann Rev Immunol*;7;145-173.
- 20 26. Kay B. Advances in Immunology: Allergy and Allergic Diseases. 2001. *N. Engl J Med*;344: 109-113.
27. Moller DR, Forman JD, Liu MC, Noble PW, Greenlee BM, Vyas P, Holden DA, Forrester JM, Lazarus A, Wysocka M, Trinchieri G, Karp C. 1996. Enhanced expression of IL-12 associated with Th1 cytokine
25 profiles in active pulmonary sarcoidosis. *J Immunol* 1996;156;4952-4960.
28. Rogge L, Papi A, Presky DH, Biffi M, Minetti LJ, Miotto D, Agostini C, Semenzato G, Fabbri LM, Sinigaglia F. Antibodies to the IL-12 receptor

- beta 2 chain mark human Th1 but not Th2 cells in vitro and in vivo. *J Immunol* 1999;162:3926-3932.
29. Gadola SD, Moins-Teisserenc HT, Trowsdale J, Gross WL, Cerundolo V. TAP deficiency syndrome. *Clin Exp Immunol* 2000;121:173-8.
- 5 30. Singh B, Read S, Asseman C, Malmstrom V, Mottet C, Stephens LA, Stepankova R, Tlaskalova H, Powrie F. Control of intestinal inflammation by regulatory T cells. *Immunol Rev* 2001;182:190-200.
31. Farrell RJ, Peppercorn MA. Ulcerative colitis. *Lancet* 2002;359:331-340.
- 10 32. Blumberg RS, Saubermann LJ, Strober W. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol* 1999;11:648-656.
33. Dohi T, Fujihashi K, Rennert PD, Iwatani K, Kiyono H, McGhee JR. Hapten-induced colitis is associated with colonic patch hypertrophy and
- 15 T helper cell 2-type responses. *J Exp Med* 1999;189:1169-1179.
34. Williams EJ, Haque S, Banks C, Johnson P, Sarsfield P, Sheron N. Distribution of the interleukin-8 receptors, CXCR1 and CXCR2, in inflamed gut tissue. *J Pathol* 2000;192:533-539.

CLAIMS

1. A method of investigating a patient's risk of developing bronchiectasis, the method comprising the step of testing the
5 genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient.
2. A method of selecting a course of treatment for a patient with or at risk of developing bronchiectasis the method comprising the step of
10 testing the genotype of genes involved in IFN γ signalling or CXCR-1 signalling of the patient.
3. The method of claim 1 or 2 wherein the patient has an immune
15 disregulation condition.
4. The method of claims 3 wherein the immune disregulation condition is ulcerative colitis.
5. The method of any of the previous claims wherein the gene involved
20 in CXCR-1 signalling is CXCR-1.
6. The method of any of claims 1 to 4 wherein the gene involved in IFN γ signalling is IFN γ .
- 25 7. The method of any of the preceding claims wherein the method comprises the step of testing the patient's genotype at IFN γ position +874.

8. The method of any of the preceding claims wherein the method comprises the step of testing the patient's genotype at CXCR-1 position +2607.
- 5 9. The method of claim 7 when dependent on claim 2 wherein when the IFN γ genotype at position +874 is A/A or A/T the course of treatment is IFN γ therapy.
- 10 10. The method of claim 8 when dependent on claim 2 wherein when the CXCR-1 genotype at position +2607 is C/C or G/C the course of treatment is CXCR-1 therapy.
11. A pharmaceutical composition comprising:
 - 15 a) an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide, or an agonist or antagonist of IFN γ ; and,
 - b) a CXCR-1 polypeptide or soluble portion thereof or a nucleic acid sequence encoding a CXCR-1 polypeptide or soluble portion thereof, or an agonist or antagonist of CXCR-1; and,
 - 20 c) a pharmaceutically acceptable excipient.
12. The pharmaceutical composition of claim 11 further comprising a therapeutically appropriate quantity of steroids or agents that suppress the immune response.
- 25 13. Use of an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or CXCR-1

polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to claim 11 or 12 in the manufacture of a medicament for the treatment of a patient with or at risk of developing bronchiectasis.

14. A method of treating a patient with or at risk of developing bronchiectasis by administering a therapeutically appropriate quantity of an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to claim 11 or 12.
15. Use of an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to claim 11 or 12 in the manufacture of a medicament for the treatment of a patient with or at risk of developing ulcerative colitis.
16. A method of treating a patient with or at risk of developing ulcerative colitis by administering a therapeutically appropriate quantity of an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to claim 11 or 12.

17. A kit of parts comprising:
- 5 i) an IFN γ polypeptide or a nucleic acid sequence encoding an IFN γ polypeptide or an IFN γ agonist or antagonist and/or a CXCR-1 polypeptide or a nucleic acid sequence encoding a CXCR-1 polypeptide or a CXCR-1 agonist or antagonist and/or a pharmaceutical composition according to claim 11 or 12; and,
- 10 ii) polynucleotides suitable for use in the method of any of the preceding claims.
18. A kit of parts comprising polynucleotides for testing the IFN γ genotype and for testing the CXCR-1 genotype.
- 15 19. Any novel method, polynucleotide, polypeptide or pharmaceutical composition as herein disclosed.

Figure 1**A****Page 1 of 4**

1 agcaaatgat caatgtgctt tgtgaatgaa gagtcaacat ttaccaggg cgaagtgggg
61 aggtacaaaa aaatttccag tccttgaatg gtgtgaagta aaagtgcctc aaagaatccc
121 accagaatgg cacaggtggg cataatgggt ctgtctcatc gtcaaaggac ccaaggagtc
181 taaaggaaac tctaactaca acacccaaat gccacaaaac cttagttatt aatacaaaact
241 atcatccctg cctatctgtc accatctcat cttaaaaaac ttgtgaaaat acgtaatcct
301 caggagactt caattaggta taaataccag cagccagagg aggtgcagca cattgttctg
361 atcatctgaa gatcagctat tagaagagaa agatcagtta agtcctttgg acctgatcag
421 cttgatacaa gaactactga tttcaacttc tttggcttaa ttctctcgga aacgatgaaa
481 tatacaagtt atatcttggc ttttcagctc tgcacgttt tgggttctct tggctgttac
541 tgccaggacc catatgtaaa agaagcagaa aacctaaga aatattttgt aagtatgact
601 ttttaatagt acttgtttgt gggtgaaaat gactgaatat cgacttgctg tagcatctct
661 gataggctgt catctcttgt aggcagtcac tttgagattt ggtgttattt tgtaattat
721 tgactagatg agttccttga ctaaataatc tagatattgt ttaaccttc tgctcagttt
781 gtatagagac ttaaaagga tttatgaatt ttccaaaaga tgggcataat atgggtatga
841 agcataatga tgtaataaat tttgtgggtg gaactcattc agttgtgata gtcaaggagt
901 atgcagattg aaaaaaatga ttgggttatta gtttttgact tctcagactc taaggtcaag
961 attagcatta aaaaggtaat aggaaatgtt taaaaattaa agtcaaaaag gtccttaaag
1021 ctttggctta aaaaaataac tgataggta ttttctcaa aaagtgattt caacattctg
1081 cttctctatc tatattactt gtgaagtatt ccggaacttc gttgctcact gggattttgg
1141 aagaattatg attctggcta agaatgttt aaaaatttta agtgaatttt ttgagtttct
1201 tttaaaattt tattgatggg taatgaaaag tttttacatt ttaaattttt cattatttgt
1261 ttaaaactta gctgttataa ttatagctgt cataataata ttcagacatt cacaattgat
1321 tttattctta caacacaaaa tcaaatctca cacacacaca cacacacaca cactcgcaca
1381 tgtttggaac tatcttttaa agctcgtata ataataccct acaggaaggc acagtagatg
1441 taatagaaac ctgtaccatt ggggggcagt attttatagt ggggtggcct tgctgttttt
1501 tgtttttgta ttttttagcc tagcttgaaa atactttctt tagcttacta tagtttttgg
1561 gacctttgga gtatcagctt tgttgagctc atttgtgaca ttgcaattta atgggtatat
1621 tgggaaataa aaaagctaaa agaacataat agtccttctc tatatctcac ataagccttt
1681 tgggaatact tattgttaga actaagcaga agagttgaaa aggaaatcag tgaatattgt
1741 cacatctgag ttcaatgaaa cttgaaatat atttttaagg caatttatgg gctaattgta
1801 aaccaatttt ttcttttttt tttttagaat gcaggtcatt cagatgtagc ggataatgga
1861 actcttttct taggcatttt gaagaattgg aaagaggtaa gctgaatatt cccatttggc
1921 taattttcct gttgcttgct ttctgatgga taaattcaca tcatcctctg tttgtgctct
1981 ttccttccaa ggagagtgc agaaaaataa tgcagagcca aattgtctcc ttttacttca
2041 aactttttta aaacttttaa gatgaccaga gcatccaaaa gagtgtggag accatcaagg
2101 aagacatgaa tgtcaagttt ttcaatagca acaaaaagaa acgagatgac ttcgaaaagc
2161 tgactaatta ttcggtgagg ctatttaaat tctttctttg gtttcattgc cgagggtcct

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2221 gcaaagcatt tattctccag aaagtagaca ttagctatatt aacagttgct aaagctatga
 2281 actcaactca tggctgaaac tctaccttac tatttccatt cgtgtttggg tgactttgca
 2341 aagccagtaa gagaatcgct gaagtatgta atgtagagaa atgctggcat tgtaactatt
 2401 gcgtaaagac aggtgagttg acaaatcca gtgaagagga agtaggtgag gaagaagcag
 2461 ggagtactga gaagcagttc tctcattgtc ccttgctcat atgatggaaa ttctcttact
 2521 ttgaatgaga ggctgtctgt cttaatggaa agagcagtgaggaggactga gaagatgtgt
 2581 gttctcctcc caactcagcc accaaggaac tgtgatgaat cacatggctg gctgggctca
 2641 gtttctctcat cttaaaagga aactgttagg ttactgtat aagtttgatg accttctttg
 2701 ctccaaaact ctacaatgca aagaatagaa aatgagaatg agatagaaga aagctacagt
 2761 ctttgaatag gtaccaggga caccctactg caagtctcta gccaacctat cagattgtac
 2821 tgcccaatta gaagcaagaa tgggtgtctgt ttgtttgttt ttagggaata atagatagaa
 2881 ttatacctt atgaaaagat tgttctatca actctctatc aactttcaga atatctcagc
 2941 tggagaactc cttagactcc taagtcttac ctcatgaact tgtatcttta agttatggct
 3001 tctataaaca gaaagataac gttgaggcat aaagacaaat catgtttttc agaatgtttt
 3061 ctagaagaca aaggcctcta gattcctttg gggttgactt tgatataaat gggctcaaat
 3121 gagagggacc aggtcttcca agctagcatt tgtgttctta ggatatgtgc tcagctttca
 3181 ctattgctgg gctgctctc cactcctctc atgtaagccc ccagaaacag aaaggagaga
 3241 catggcaaca ggtctccttt ggttataaac tagacactca gcacttgttt ctaatccagt
 3301 ggtgcccctg gcttactgtt cagtcttggg taagtctctt agtttcttgg tgatgatttg
 3361 aacattggaa agtaaaatct gtcacttgca aacacacagc ttgtcgaaaa ttttttctac
 3421 tctgcaggaa ctgggcctta aaaaaatgaa aaaaaatctg tggtttcttc cttctggaag
 3481 ctacaaacct cctgtttctt gatgggcaat cttgagttag ctctattaat tattattctc
 3541 tttggctcag ttgctaagct attttatgca tgttatgccc tttgacaatt agtcttttagc
 3601 tgtaatcccc cagccatcct cagaaatgtg gtgaggtagc catagtgttc ccaagattag
 3661 aaaaatgtaa tggcagagcc aagaggaagg taaatgggtcc acatcttatg aagcatcatc
 3721 taaatggccc tattgggttag agtgaggaga tgcaagtagt tcaatttgct tgcttagaag
 3781 gcagggtagt ggaaaagttg ttgcaattct taattttaaa ctttatatat cagtaagcca
 3841 tatataaata tgattggggg tgtttatatt aaaatctatt atggaaattg agagactgac
 3901 ctaatctggg agaaattaaa aattacagtt ttactcgtt ttggatttgg tgttttctag
 3961 ggtacctaac cttagatcagt ggttctcaaa cttaggtgga tgtcagaatc acctggggag
 4021 cttagtgaat gcacagggca cagtcttcc acttcatgca cctggatctc tgaggctctt
 4081 gacaggtttc cggattaatc tgctatgcac aacagtgaga atcattgacc tatagttact
 4141 catttgatgc atacaggaaa gactgaagta taaagtgata taattggtag attgatgata
 4201 gagaggatcat agaaacagtc tcatcctcct ttagatgaga aaatagaagt tcagagagg
 4261 taagtagctg gctcaaggtc agaattattg catgcatgag attcaaacc acctttttat
 4321 gctgactcca caaccaggag tcttttcaat atataatttc aagaattcta tagaagtaga
 4381 tttaaagata tgtgatggac tccaccacat tatagcaca ctagaaatgt aattgtaatt
 4441 tttagcttca actgctgaag aagtaaatat tgtatatata ggtaatacgg tccatttttt
 4501 aaaggaatac ttttattttc actgaccatc atgacattag cagaatatcc tgatggctta

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4561 tatgcctgaa attaatTTTTg ctcttttctt tcccgatagg taactgactt gaatgtccaa
4621 cgcaaagcaa tacatgaact catccaagtg atggctgaac tgtcgccagc agctaaaaca
4681 gggaagcgaa aaaggagtca gatgctgttt cgaggctgaa gagcatocca gtaatggttg
4741 tcctgcctgc aatatttgaa ttttaaactc aaatctattt attaatattt aacattattt
4801 atatggggaa tatattttta gactcatcaa tcaaataagt atttataata gcaacttttg
4861 tgtaatgaaa atgaatatct attaatatat gtattattta taattcctat atcctgtgac
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4981 tatctcaggg gccaaactagg cagccaacct aagcaagatc ccatgggttg tgtgtttatt
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5941 aagcaaaaaca gccaaaggatc c

Figure 1**Page 4 of 4****B**

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Figure 2**Page 1 of 5****A**

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301    aacaaatatg ctcacttttc aaggctctct ctggcttctc tggagactgg agatcagggc
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1741   agggccccgt cttttttctc ctgtgtcccc agtgactatt ttctgccctt ggggtgatttt
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2101   cagtatatca ggacctttc tctacaaata aataaataac taaataaaaa ataaaaataa
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Figure 2
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Figure 3

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Originator	Generic Name	World Status	Any Therapy Code	Any Therapy Description
Advanced Viral Research	Product R	Phase II Clinical Trial	T4E J5A J5Z A14 K5B M2C A10B	Non-antisense oligonucleotides Antiviral, anti-HIV Antiviral, other Anabolic Radio/chemoprotective Antiarthritic, immunological Antidiabetic
Amgen	interferon, Amgen (Gamma)	Discontinued	T2A1 K4 M2C	Recombinant interferon Anticancer, interferon Antiarthritic, immunological
Amgen	macrophage activator, Immunex	Discontinued	T2A3 I1Z	Recombinant growth factor Immunostimulant, other
Asahi Kasei	interferon, Asahi (Gamma)	Discontinued	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
Aventis	interferon, Behr (Alpha, β , Gamma)	No Development Reported	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
Biogen	interferon, Biogen (Gamma)	Launched	T2A1 K4 I2	Recombinant interferon Anticancer, interferon Cytokine
Celltech	macrophage activator, Celltech	No Development Reported	T2A3 I1Z	Recombinant growth factor Immunostimulant, other

Figure 3

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Cellular Products	interferon, Cellular Prod (Gamma)	Discontinued	K4 J5B I2	Anticancer, interferon Antiviral, interferon Cytokine
Chiron	interferon gene ther, Chiron V	No Development Reported	T4A K4	Gene therapy Anticancer, interferon
CJ Corp	interferon, CJ Corp (Gamma)	Discontinued	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
Gedeon Richter	thymocartin	Discontinued	I1Z M1A1	Immunostimulant, other Anti-inflammatory
GlaxoSmithKline	interferon, GlaxoSmithK1 (Alpha2)	Discontinued	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
Hayashibara	interferon, Hayashibara (Gamma)	Launched	K4 I2	Anticancer, interferon Cytokine
ICN	interferon, Flow (Gamma)	No Development Reported	K4 J5B I2	Anticancer, interferon Antiviral, interferon Cytokine
Immuno Modulators	interferon, IML (Gamma)	Discontinued	K4 J5B I2	Anticancer, interferon Antiviral, interferon Cytokine
Imreg	imreg-1	No Development Reported	I1A I1Z K3 J4A	Immunostimulant, anti-AIDS Immunostimulant, other Anticancer, immunological Antimycobacterial
Interferon Sciences Kaneka	interferon, ISI (Gamma) interferon, Kaneka	No Development Reported Discontinued	K4 T2A1 K4 J5B I2	Anticancer, interferon Recombinant interferon Anticancer, interferon Antiviral, interferon Cytokine

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Figure 3

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La Jolla Pharmaceutical	macrophage activator, La Jolla	Discontinued	T2A3 I1Z J7B K3	Recombinant growth factor Immunostimulant, other Immunomodulator, anti-infective Anticancer, immunological
LG Life Sciences	interferon, LGLS	Launched	T2A1 K4 M2C I2	Recombinant interferon Anticancer, interferon Antiarthritic, immunological Cytokine
Ligand	transcription factors, Ligand	No Development Reported	T2Z I6Z K3 J7B	Recombinants, other Immunological Anticancer, immunological Immunomodulator, anti-infective
Maxygen	interferon, Maxygen (Gamma)	Preclinical	T2A1 I2 R9Z K4 R4A J3Z M2C K1A A10C S1Z	Recombinant interferon Cytokine Respiratory Anticancer, interferon COPD treatment Antibacterial, other Antiarthritic, immunological Anticancer, antibiotic Symptomatic antidiabetic Ophthalmological
Mercian	cytogenin	Discontinued		
Mitsubishi Pharma	interferon, Mitsubishi (Gamma)	Discontinued	J5B K4 I2	Antiviral, interferon Anticancer, interferon Cytokine
Non-industrial source	apurinic acid	No Development Reported	J5A	Antiviral, anti-HIV

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Figure 3

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Non-industrial source	Pharmaprojects No. 4745	No Development Reported	T4A K3 K4	Gene therapy Anticancer, immunological Anticancer, interferon
Novacell	Gamma-interferon, Novacell	Discontinued	F9Z9 I1Z K3 T3A9	Formulation, other Immunostimulant, other Anticancer, immunological Monoclonal antibody, other
Novaferon	interferon, Novaferon (Gamma)	No Development Reported	J5B K3 I2	Antiviral, interferon Anticancer, immunological Cytokine
Novartis	interferon, Novartis (Gamma)	Discontinued	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
OM PHARMA	OM-174	Phase I Clinical Trial	K3 I1Z	Anticancer, immunological Immunostimulant, other
Pfizer	interferon, Pfizer	Discontinued	T2A1 K4 J5B I2	Recombinant interferon Anticancer, interferon Antiviral, interferon Cytokine
Pfizer Rentschler Sclavo	malaria vaccine, Kabi interferon, Rentschler (Gamma) interferon, rDNA, Sclavo (Gamma)	Discontinued Discontinued Discontinued	J7A1 I2 T2A1 K4 J5B I2	Prophylactic vaccine Cytokine Recombinant interferon Anticancer, interferon Antiviral, interferon Cytokine

Figure 3

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Sheffield Pharmaceuticals	interferon, Sheffield (Gamma)	Preclinical	F1B1 J7B I2	Formulation, inhalable, systemic Immunomodulator, anti-infective Cytokine
Shionogi Sidus	MAF, Shionogi interferon, Sidus (Gamma)	No Development Reported No Development Reported	I1Z T2A1 K4 P1Z	Immunostimulant, other Recombinant interferon Anticancer, interferon Parasiticide
SR Pharma	SRL-172	Phase III Clinical Trial	K3 J7B R8B R8A D4A I1A D5A	Anticancer, immunological Immunomodulator, anti-infective Antiallergic, non-asthma Antiasthma Antipruritic/inflamm, allergic Immunostimulant, anti-AIDS Antipsoriasis
SRC VB VECTOR	Gamma-interferon analogues, VECT	No Development Reported	T2A1 J5B K4	Recombinant interferon Antiviral, interferon Anticancer, interferon
Takeda	Gamma-interferon/interleukin-2	No Development Reported	T2A1 T2A2 I2	Recombinant interferon Recombinant interleukin Cytokine
Takeda	interferon, Takeda (Gamma)	Discontinued	T2A1 K4 J5B I2	Recombinant interferon Anticancer, interferon Antiviral, interferon Cytokine

Figure 3

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Theracel	ketobutyrolactones	Discontinued	K3 I1A I1Z	Anticancer, immunological Immunostimulant, anti-AIDS Immunostimulant, other
Transgene	Ad-JFNGamma	Phase II Clinical Trial	T4A K4	Gene therapy Anticancer, interferon
Transgene	interferon, Transgene (Gamma)	No Development Reported	T2A1 K4 P1G I2	Recombinant interferon Anticancer, interferon Protozoacide Cytokine
Transgene	interferon-Gamma gene therapy, Tr	No Development Reported	T4A K4	Gene therapy Anticancer, interferon
Virax	AIDS vaccine, Virax	Phase II Clinical Trial	T2B J7A2	Recombinant vaccine Therapeutic vaccine
Yeda	interferon, Yeda (Gamma)	No Development Reported	T2A1 J5B K4 I2	Recombinant interferon Antiviral, interferon Anticancer, interferon Cytokine
Yissum	interferon, Yissum (Gamma)	No Development Reported	T2A1 K4 J5B I2	Recombinant interferon Anticancer, interferon Antiviral, interferon Cytokine

Figure 4

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Originator	Generic Name	World Status	Any Therapy Code	Any Therapy Description
Advanced Biotherapy	AGT-1	Phase I Clinical Trial	T3A9	Monoclonal antibody, other
			M2C	Antiartbritic, immunological
			N7A	Multiple sclerosis treatment
			I5	Immunosuppressant
			J5A	Antiviral, anti-HIV
Fujimoto Pharmaceutical Hoffmann-La Roche	LK-423 Pharmaprojects No. 2697	No Development Reported	S1Z	Ophthalmological
			A10B	Antidiabetic
			D5A	Antipsoriasis
Innogenetics	anti-IFN-Gamma MAb, Innogenetics	Discontinued	M1A1	Anti-inflammatory
			T2Z	Recombinants, other
			M1A1	Anti-inflammatory
			I5	Immunosuppressant
Johnson & Johnson	Gamma-interferon antibody, Cent	No Development Reported	T3A5	Monoclonal antibody, humanized
			A14	Anabolic
			T3A2	Monoclonal antibody, human
			N7A	Multiple sclerosis treatment
Merck & Co	simvastatin	Launched	C10	Hypolipaeic/Antiatheroscler
			C9Z	osis
			M2Z	Cardiovascular
				Antiartbritic, other

Figure 4

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Protein Design Labs	fontolizumab	Phase II Clinical Trial	T3A5 A16 D5A N7A A10B M2C	Monoclonal antibody, humanized GI inflammatory/bowel disorders Antipsoriasis Multiple sclerosis treatment Antidiabetic Antiarthritic, immunological
Schering-Plough	Gamma-interferon inhibitors, Sch	No Development Reported	T2Z I5	Recombinants, other Immunosuppressant

Figure 5

Originator	Generic Name	World Status	Any Therapy Code	Any Therapy Description
Celltech	CXCR2 antagonists Celltech	No Development Reported	M1A1 D5A M2C R9Z	Anti-inflammatory Antipsoriasis Antiarthritic, immunological Respiratory
Dompe	repertaxin L-lysine salt	Phase I Clinical Trial	C9Z A5B N7C G4Z A16 D4A	Cardiovascular Hepatoprotective Neuroprotective Urological GI inflammatory/bowel disorders Antipruritic/inflamm, allergic
Fournier	LF-216	No Development Reported	R4A M2Z A16	COPD treatment Antiarthritic, other GI inflammatory/bowel disorders

Figure 6

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Originator	Generic Name	World Status	Any Therapy Code	Any Therapy Description
Abgenix	ABX-IL8	Discontinued	T3A2	Monoclonal antibody, human
			D5A	Antipsoriasis
			R4A	COPD treatment
Amgen	interleukin-8 receptor, Immun	No Development Reported	T2Z	Recombinants, other
			M2C	Antiarthritic, immunological
			I5	Immunosuppressant
Aventis	interleukin-8 antags, Select	No Development Reported	R8A	Antiasthma
Aventis	pentoxifylline, HIV	Discontinued	I5	Immunosuppressant
			J5A	Antiviral, anti-HIV
			A14	Anabolic
BTG	LS-4249	Discontinued	N6D	Cognition enhancer
			J5Z	Antiviral, other
			M1A1	Anti-inflammatory
Celera	CT-8	Discontinued	R9Z	Respiratory
Genomics	CXCR2 antagonists, Celltech	No Development Reported	R4B	Cystic fibrosis treatment
			M1A1	Anti-inflammatory
			D5A	Antipsoriasis
CytRx	CRL-1072	No Development Reported	M2C	Antiarthritic, immunological
			R9Z	Respiratory
			J4A	Antimycobacterial
Dainippon	interleukin-8, Dainippon	No Development Reported	P1G	Protozoacide
			J5Z	Antiviral, other
			T2A2	Recombinant interleukin
Fournier	LF-216	No Development Reported	I2	Cytokine
			R4A	COPD treatment
			M2Z	Antiarthritic, other
Genentech	interleukin-8, Genentech	No Development Reported	A16	GI inflammatory/bowel disorders
			I2	Cytokine
			I6Z	Immunological
GlaxoSmithKlin	Pharmaprojects No. 4269	No Development Reported	M1A1	Anti-inflammatory
GlaxoSmithKlin	Pharmaprojects No. 5568	No Development Reported		

Figure 6

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GlaxoSmithKline	SB-265610	No Development Reported	M1A1 C10 R9Z	Anti-inflammatory Hypolipaeamic/Antiatherosclerosis Respiratory
Japan Tobacco Kosan Biosciences	Pharmaprojects No. 5502 macrolide analogues, Kosan	No Development Reported No Development Reported	M2C R4A R8A N11Z T3A2 I5	Antiarthritic, immunological COPD treatment Asthma Neurological Monoclonal antibody, human Immunosuppressant
Millennium	anti-IL-8 MAb, Millennium	No Development Reported	I5	
Millennium Non-industrial Non-industrial Novartis	IL-8 antagonists, Millennium antileukinate Pharmaprojects No. 3963 interleukin-8, Novartis	No Development Reported No Development Reported No Development Reported No Development Reported	C9Z K3 R9Z I2 D11Z	Cardiovascular Anticancer, immunological Respiratory Cytokine Dermatological Antiarthritic, other Asthma Respiratory Cardiovascular Anticancer, other GI inflammatory/bowel disorders
Pharmacoepia	IL-8 antagonists, Pharmacoepia	No Development Reported	M2Z R8A R9Z C9Z K6Z A16	Respiratory Cardiovascular Anticancer, other GI inflammatory/bowel disorders
Rentschler RepliGen	pentoxifylline, Rentschler IL-8 receptor, RepliGen	Discontinued No Development Reported	R9Z T2Z I5 M2C D5A B6A R8A T3A9 M1A1 C9Z	Respiratory Recombinants, other Immunosuppressant Antiarthritic, immunological Antipsoriasis Septic shock treatment Asthma Monoclonal antibody, other Anti-inflammatory Cardiovascular
Tanox	TNX-224	Preclinical		

Figure 6

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Toyama	iguratimod	Phase III Clinical Trial	M2Z	Antiarthritic, other
Yes Biotech	anti-IL-8 MAb, cancer, Yes	Preclinical	N2Z	Analgesic, other
Yes Biotech	anti-IL-8 MABs, Yes Biotech	Registered	T3A9	Monoclonal antibody, other
			K3	Anticancer, immunological
			T3A9	Monoclonal antibody, other
			D5A	Antipsoriasis
			D4A	Antipruritic/inflamm, allergic