DISSERTATION

Mechanism of action of intravenous immunoglobulins in Multiple Sclerosis
Studies of gene expression profiles in peripheral T cells

Ausgeführt zum Zweck der Erlangung des akademischen Grades eines Doktors der Naturwissenschaften unter der Leitung von

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"From the brain and the brain alone arise our pleasures, joys, laughter and jests, as well as our sorrows, pains and griefs"
Hippocrates

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Zusammenfassung

Polyspezifische humane IgG Produkte (Intravenöses Immunglobulin, IVIG) werden erfolgreich bei der Behandlung von zahlreichen Autoimmunerkrankungen, die das zentrale Nervensystem betreffen, eingesetzt. Ein relativ neues Anwendungsgebiet für IVIG in diesem Sektor ist Multiple Sklerose (MS).

Obwohl IVIG einen substantiellen Effekt bei Kurzzeit- und Langzeitbehandlungen zeigt, sind die genauen Wirkungsmechanismen noch weitgehend unbekannt.

Der positive Effekt der IVIG Behandlung könnte durch eine Veränderung der T-Zell-Antwort im Zuge der Immunantwort verursacht werden.

Deshalb war das Ziel meiner Dissertation die Identifizierung und Charakterisierung von Genen, die in den immunmodulatorischen Altivitäten von IVIG bei der Behandlung von Schüben bei Patienten, die an rezidivierend-remittierender MS (RRMS) leiden, involviert sind.

Mit Hilfe von Microarrays konnten wir die Expressions-Profile von T-Zell-Fraktionen untersuchen. Die T-Zellen wurden aus peripheren Blut-Mononucleären Zellen (PBMC) der 10 Patienten, die an unserer klinischen Studie teilgenommen haben, isoliert. Zur Kontrolle wurde eine Gruppe von 5 Patienten mit intravenösem Methylprednisolone (IVMP) behandelt. Unter den 22.000 Genen, die auf dem Chip lokalisiert sind, fanden wir 152 verschiedenen Gene oder 176 verschiedenen Probe-Sets, die zumindest in 40% der Patienten mindestens 2-fach verändert waren und somit differentiell expremiert wurden. Die meisten der Proteine, die durch die Gene codiert werden, spielen eine Rolle in Immunantwort, Entzündungserscheinungen, Proliferation, Apoptose, Zellzyklus, Signaltransduktion oder Regulierung von Transkription. All diese biologischen Aktivitäten könnten mit der Regulation der Krankheits-Aktivität in Patienten, die an RRMS leiden, assoziiert sein.

Zur Verifizierung der Microarray-Daten wurde ebenfalls eine statistische Untersuchung des Datensatzes durchgeführt, bei der die Daten mit Hilfe eines parametrischen t-tests analysiert wurden. Dieser Ansatz ergab eine gänzlich unterschiedliche Anzahl an differentiell expremierten Genen. Ein Vergleich der zwei verschiedenen Ansätze ergab nur eine geringe Zahl an Genen, die in beiden Methoden gleich verändert waren. Diese Unterschiede in den Ergebnissen der Genespressions-Studie ist ein bekanntes Problem in der Literatur. Um

reproduzierbare und vergleichbare Expressions-Daten einer Microarray-Analyse zu bekommen, ist eine internationale Standardisierungs-Richtlinie nötig.

Zusammenfassend glauben wir, durch diese zwei Methoden Gen-Sets gefunden zu haben, die bei der biologischen Aktivität von IVIG bei der Behandlung von Patienten, die an einem akuten MS-Schub leiden, eine zentrale Rolle spielen.

Abstract

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of autoimmune diseases of the central nervous system including multiple sclerosis (MS).

Although IVIG seems to have a substantial effect on short- and long-term treatment potential, the underlying mechanisms of action are not elucidated.

The benefical effects of IVIG treatment might be caused by a modulation of the T cell immune response. Therefore, the aim of my PhD-thesis was the identification and characterization of genes involved in the immunomodulatory activity of IVIG in the treatment of exacerbations in Relapsing-Remitting MS (RRMS).

Using microarrays we investigated the expression profiles of T cell fractions of peripheral blood mononuclear cells (PBMC) isolated from 10 RRMS patients treated with IVIG as well as five control patients treated with intravenous methylprednisolone (IVMP). Among the approximately 33.000 genes examined, we found 152 different genes (176 probe-sets) which were differentially regulated by a minimum of a two-fold change in at least 40% of patients. Most of the proteins encoded by these genes are known to be involved in immune response, inflammatory response, proliferation, apoptosis, cell cycle, signal transduction or regulation of transcription. All these biological activities might be associated with the regulation of disease activity in patients with RRMS. Statistical analysis by parametric t-test revealed a different number of significantly differentially regulated genes. When comparing the results obtained with both approaches, only a few genes were in common. Differences in the results obtained from gene expression profiles using different approaches for the evaluation of the data are a known problem in the literature. International standartization of statistical approaches for the evaluation of gene expression data obtained from microarray analysis will be necessary to generate reproducible and comparable results in different laboratories.

In conclusion we believe to have identified two set of genes by using two different methods – a non statistical and a statistical approach - that are likely involved in the biological activity of IVIG in patients suffering from acute exacerbations.

INTRODUCTION

1.1 Intravenous Immunoglobulins (IVIG)

1.1.1 Introduction

Immunoglobulins are proteins produced by cells of the B lymphocyte lineage of the immune system and are the major effector molecules of the humoral immune response.

Intravenous immunoglobulins (IVIG) are prepared from plasma pools of 15.000-50.000 healthy donors and contain > 97% of intact IgG molecules and, depending on the product, small traces of IgM and IgA molecules. The IgG subclasses are distributed similar to normal serum.

Therefore, IVIG comprises a broad range of immune antibodies directed to pathogens and foreign antigens that are essential for substitution-treatment of patients with primary and secondary immune deficiencies.

Primary immunodeficiency disorders are a group of genetic diseases in which the body is unable to produce adequate amounts of its own antibodies and is at increased risk of severe and partially life-threatening infections. Among these diseases there are agammaglobulinemia and hypoglobulinemia, common variable immunodeficiency (CVID), severe combined immunodeficiencies (SCID) and Wiskott-Aldrich syndrome.

Secondary immunodeficiencies are disorders in which the patients are unable to produce antibodies due to another underlying condition, and they suffer from severe infections. Among these diseases there are myeloma or chronic lymphocytic leukemia, children with congenital AIDS and recurrent infections, bone marrow and other transplantations in which chemotherapy can result in a period of immunodeficiency.

IVIG has proven efficiency in controlled clinical trials for the treatment of autoimmune thrombocytopenic purpura, Guillain Barrè syndrome, chronic inflammatory demyelinating polyneuropathy, acute myasthenia gravis, multifocal

motor neuropathy, steroid-resistant dermatomyesitis, autoimmune uveitis, Kawasaki syndrome and ANCA-associated vasculitides (1).
Pharmacokinetics of most IVIG preparations reflect metabolic properties of normal IgG. After infusion in normal individuals and patients, serial determinations of total IgG result in biphasic plasma or serum disappearance curves with an initial α phase, which represents early catabolism and distribution between body compartments, and a final β phase representing catabolism.
In immunologically normal persons the half-life values of IVIG preparations were between 14 – 24 days, while those of various IgG antibodies were between 12 – 35 days (A).
In general there was a prolonged half-life of infused IgG in patients with congenital humoral immunodeficiencies (A).

1.1.2 History of IVIG

Immunotherapy was started at the Charitè Hospital in Berlin more than 100 years ago with the administration of diphtheria antitoxin sera of animal origin under the supervision of von Berhring, Ehrlich and Kitasato from the Robert Koch Institute.

Initially, immunoglobulin was used as a prophylaxis or treatment against measles, tetanus, diphtheria, hepatitis B and pertussis. In 1952 for the first time immunoglobulin preparations from human blood were used in clinical medicine to treat immune deficiency conditions. The only available preparations at that time required intramuscular administration (IMIG). But intramuscular administration was painful for the patient, muscle proteases degraded many of the infused immunoglobulins and the remaining IGs only reached the circulation after delay. The injections were also limited in dose and frequency.
Nowadays there are preparations for intravenous administration available. Initially IVIG preparations were used for substitution of immune deficiencies but they are now used as therapeutic and prophylactic reagents, too.

Because the concentration of any single antibody in a normal IVIG preparation is relatively low, high doses are required to be clinically effective.

The process of fractionating large volumes of human plasma was developed by E.J. Cohn in the 1940s in the Department of Physical Chemistry at Harvard Medical School. Originally, the Cohn fractionation procedure was developed to produce albumin solutions as blood substitution during World War II. But it has proven to be useful in large-scale separation of other classes of therapeutic plasma proteins.

This cold ethanol fractionation process which was developed by Cohn is used to produce three protein fractions: an IgG concentrate, an intermediate in the production of coagulation factors VII and IX, and human serum albumin, the only fraction which does not require additional purification steps. The process might produce an IgG fraction with a purity of > 97% (w/w).

IgG produced by Cohn ethanol fractionation was historically freeze-dried to remove ethanol and to produce a stable intermediate fraction. But this promotes the formation of IgG aggregates at the expense of monomeric IgG.

In 1962 the formation of IgG aggregates which lead to spontaneous complement activation was proposed as the principal cause of adverse side effects when intramuscular immunoglobulin (IMIG) was injected intravenously. Therefore anticomplement activity hemolytic tests became routinously used.

From this time on commercial IVIG preparations tended to reduce the anticomplement activity, either by enzymatic digestion or chemical modification.

In 1986 McCue and co-workers developed stable IVIG solutions by adjustment of the pH (4).

Cold ethanol fractionation has been considered as a sufficient process for the elimination and inactivation of viruses due to high concentrations of ethanol at low temperature. In the 80s plasma pools were contaminated with HIV and patients treated for hemophilia with other blood products made out of the same pool, were transmitted with AIDS.

Today IVIG preparations are manufactured in accordance with standardized safety measures to ensure a maximum of safety, beginning with strict selection of only highly qualified donors and prescreening and screening for viruses by PCR. Donations containing high titers of human parvovirus B19 nucleic acids, HIV, HCV or HBV are rejected. The plasma is also screened for alanine aminotransferase

(ALT) levels, a measurement for liver function. All plasma units are held for at least 60 days and released only after the donor returns for subsequent donation of plasma. This procedure ensures that a donor infected by a certain pathogen, but does not show any clinical symptoms at the time of donation, is identified by the subsequent testing and screening.

The manufacturing process of, for example Endobulin S/D includes plasma collection with HIQ-PCR testing of plasma pilot pools and plasma production pools for HIV-1, HIV-2, HBV and HCV, Cohn-Oncley Fractionation for isolating the immunoglobulins from coagulation factors and inhibitors, DEAE-Sephadex chromatography for IgA depletion - yielding a product with a high level of monomeric and dimeric IgG and virus removal against some lipid (HIV, pseudo rabies virus PRV) and all investigated non-lipid enveloped viruses (HAV, MMV), sovent/detergent treatment for viral inactivation of all lipid-enveloped viruses tested to date (HIV, HCV, HBV) by incubating fraction II with solvent tri(n-butyl) phosphat (TNBP) and the detergents Triton X-100 and Tween 80, removal of the solvent/detergent by binding of IgG to an electrostatic matrix while the rest of the either negatively or neutral chemicals is washed away, incubation of fraction II with hydrolases at 37° for the inactivation of vaso active substances, PEG precipitation with polyethylene glycol for removal of aggeregates which may have been formed during incubation, and finally sterile filtration and addition of stabilizing agents like sodiumchloride and glucose to preserve antibody activity and prevention of aggregation of the monomeric IgG and freeze drying.

Open-label studies of pharmacokinetics and tolerance in primary immunodeficiency patients showed no occurrence of virus transmission, a low number and no serious adverse side effects. The risk of adverse/anaphylactic reactions is minimized by testing for parameters known to cause reactions. Furthermore, there are also animal models to assess the potential for adverse events caused by hypotensive and bronchospastic substances (like aggregates).

All these actions taken today are to ensure maximal safety and tolerability for patients.

1.1.3 Mode of action of IVIG

IVIG preparations have shown to be effective in the treatment and prophylaxis of infectious complications in patients with primary or secondary humoral immunodeficiencies as well as in the treatment of various autoimmune diseases. But the precise mechanism of action underlying these immunomodulatory activities has not been elucidated.
There are at least four models to explain the immunomodulatory potential of IVIG in patients suffering from autoimmune and inflammatory diseases:

I. Fcγ-Receptor-Mediated Immunomodulation
II. Influence on Idiotype/Anti-Idiotype Network
III. Elimination of Immunostimulating Microbial Products
IV. Immunomodulatory Effects of Ig-associated Molecules

I. Fcγ-Receptor-Mediated Immunomodulation

IG preparations available on the market mainly consist of IgG (in particular IgG1 and IgG2). IgG1 especially can bind via the Fc part not only to Fc receptors I-III (CD64, CD32, CD16) but also to C1q. IgG1 is also able to bind to cell-bound complement receptors (CD11b, CD11c) by activation and binding of other complement factors, but the best complement activator is IgG3 which is only contained to a small amount in IVIG preparations. IgG1 and IgG3 bind with high-affinity to FcγRI expressed on macrophages, neutrophils and also eosinophils.
In general, there are three distinct FcγR classes: FcγRI, FcγRII and FcγRIII with different IgG class specificities and binding affinities. The only high affinity receptor due to its third extracellular domain is FcγRI (CD64) with its specifity for IgG1 and IgG3. It can bind monomeric and aggregated Ig and functions mainly in phagocytosis. FcγRII (CD32) is a low affinity receptor which binds IgG in the form of immune complexes with a specificity for IgG1 and IgG3. FcγRIIA is mainly expressed by macrophages and neutrophils and functions in phagocytosis and cell activation while FcγRIIB is mainly expressed by B lymphocytes and functions in feedback inhibition of B cells. FcγRIII (CD16) bind immune complexes with low to

medium affinitiy. FcγRIIIA is expressed by Natural Killer cells (NK) cells and functions in ADCC, FcγRIIIB is expressed by neutrophils and functions in phagocytosis (2).

As Fcγ and complement receptors are mainly expressed by immune cells like B cells, T cells, NK, monocytes/macrophages, dendtritic cells and granulocytes, activation can lead to intracellular signaling cascades resulting in immunomodulatory consequences.

Fc receptors can bear either activation (ITAMs) or inhibiting (ITIMs) motifs in their intracytoplasmic domains. Especially the FcyRIIB receptor possesses such an inhibitory signal. It is a low-affinity receptor binding immune complexes.(3) Antigen-IgG antibody complexes are thus able to inactivate B lymphocytes and inhibit differentiation in IG producing plasma cells through a negative regulation via their ITIMs. This negative feedback reaction might be an explanation for the IG-induced inhibition of autoantibody synthesis (B).

IG preparations can contain anti-idiotypic antibodies and thus have an influence on the idiotype/anti-idiotype network (B). Therefore, therapeutic IG antibodies bind directly to (auto-)antigen receptors and then also to the Fcγ receptors of B lymphocytes (B) which may lead to negative regulation of B cell proliferation in response to the inhibitory signals mediated by ITIMs through binding to the FcyRIIB receptor.

There is also experimental evidence that IgG-containing complexes can modulate the cytokine production of monocytes/macrophages through binding to the Fcγ receptor IIA. In addition there seems to be an indirect inhibition of T cell function, which might explain the anti-inflammatory effect seen after IG administration, that could be due to a latent association with TGF-β. This binding can occur directly in antibody-producing B cells and also in plasma. IgG-TGF-β complexes can inhibit the activation of inflammatory TH1 cells and $CD8^+$ cells (4).

Finally, antigen presentation can be influenced by the formation of immune complexes. Antigens in these complexes are increasingly eliminated by scavenger cells like macrogphages resulting in reduction of presentation on dendritic cells.

II. Influence on Idiotype/Anti-Idiotype Network

It has been demonstrated by several investigators that the sera of healthy people contain anti-idiotypic antibodies binding to idiotypic autoantibodies.

III. Elimination of Immunostimulating Microbial Products

Neutralization of inflammation-inducing microbial products may also be the cause of the anti-inflammatory effects caused by IG. When bacterial superantigens (e.g.SEB) are administered, an inflammatory reaction through oligoclonal T cell stimulation leads to septic shock. Specific anti-SEB hyperimmune globulin preparations inhibit this inflammatory reaction by neutralization of bacterial toxins. So the efficiency of IG preparations in inflammatory conditions could be determined by the content of neutralizing toxin (superantigen) antibodies.

IV. Immunomodulatory Effects of Ig-associated Molecules

Mouse studies have shown that IgG is often associated with latent TGF-β. This binding happens directly in IgG-producing B cells and also in plasma. These IgG-TGF-β complexes can inhibit the activation of inflammatory TH1 cells and cytotoxic T cells.

1.2 *Autoimmunity*

Diseases caused by a failure of self-tolerance and subsequent pathologic immune responses against self are called autoimmune diseases.
Autoimmune diseases are classified into three subgroups according to the type of immune response, and nature and location of the antigen target.

I. Antibody mediated diseases
II. Immune complex mediated diseases
III. T cell mediated diseases

I. Antibody mediated diseases

The pathologic mechanism underlying this type of autoimmunity are IgG and IgM molecules directed against the cell surface or extracellular matrix antigens.
This leads to opsonization and phagocytosis of cells, complement- and Fc-receptor mediated recruitment and activation of leukocytes (neutrophils, macrophages) leading to an enormous immune response. Also impaired cellular functions, e.g. hormone receptor signaling might be a result.
Examples for antibody mediated disorders include autoimmune thrombocytopenic purpura, acute rheumatic fever and myasthenia gravis, where self-antibodies to the acetylcholine receptor inhibit the binding of acetylcholine followed by a down-modulation of the receptor by antibodies which leads to symptoms like muscle weakness and paralysis (B).

II. Immune complex mediated diseases

Here immune complexes of circulating antigens as well as IgG and IgM antibodies are the mechanisms resulting in complement- and Fc-receptor-mediated recruitment and activation of leukocytes resulting in inflammation, and injury to the vessels and the adjacent tissues.

Many systemic immunologic diseases are caused by this deposit of immune complexes in blood vessels, e.g. systemic lupus erythemathodes (SLE).

In SLE pathogenic T Helper (TH) cells seem to react with peptides derived from nucleosomal proteins. Self-DNA specific B cells bind nucleosomal protein-DNA complexes leading to the production of anti-DNA antibodies via T cell activation.

The immune complexes cause inflammation and complement activation in various tissues throughout the human body. Many patients show low numbers of suppressor T cells, suggesting a loss of immune tolerance due to decreased inhibition by suppressor cells (C) (5-7).

III. T cell mediated diseases

T lymphocyte can cause injury either by triggering delayed type hypersensitivity reactions (DTH) or by directly killing target cells.

$CD4^+$ T cells mediate macrophage activation and cytokine-mediated inflammation resulting in DTH reactions while CD8+ cells directly cause cell lysis

(T cell-mediated cytolysis) or also cytokine-mediated inflammation.

Examples for disorders caused by T cells are insulin-dependent diabetes mellitus (IDDM), rheumatoid arthritis and the animal model of multiple sclerosis, experimental autoimmune encephalitis (EAE), a neurologic disease where autoimmune T cells directed against myelin lead to destruction and neurologic deficits in the brain.

Therefore, also multiple sclerosis, the human equivalent to EAE might be a T cell mediated autoimmune disease (C)(8-10).

Autoimmune diseases are also caused by a (partially strong) genetic predisposition. For instance a concordance rate of 35%-50% in monozygotic twins for IDMM. Among the genes strongest associated with autoimmunity are the genes within the MHC locus, especially the HLA genes in the MHC II locus.
Finally, viral and bacterial infections are discussed to contribute to the development or exacerbation of autoimmune diseases through enhanced expression of costimulators in tissues and cross-reactions between the pathogenic antigens and self antigens.

1.3 Multiple Sclerosis

1.3.1 The Human Brain

Multiple Sclerosis (MS) is an autoimmune disease affecting the Central Nervous System (CNS).

The CNS consist of the brain and the spinal cord, immersed in the cerebrospinal fluid (CSF). The brain itself consists of three parts, the cerebrum, the cerebellum and the brainstem.

The cerebrum is divided into two hemispheres (left and right). Each consists of four lobes (frontal, parietal, occipital and temporal). The outer brain layer, or the cerebral cortex, is also known as "grey matter". It covers the nuclei which lie in the so-called "white matter", in the cerebral hemisphere.

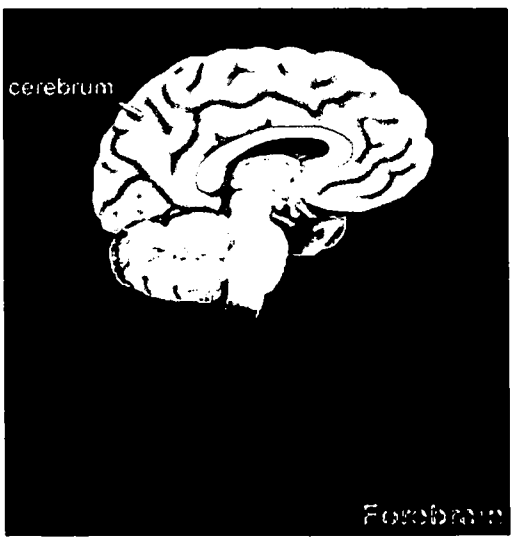

Fig. 1 The cerebrum
The main portion of the brain, made up of two cerebral hemispheres united by the corpus callosum, forming the largest part of the CNS. (www.brainexplorer.org)

The grey matter, formed by neurons, includes regions of the brain involved in muscle control and sensory perceptions.

The white matter or diencephalons is situated between the brainstem and the cerebellum. It is a neuronal tissue containing myelinated axons.
White matter nuclei are involved in the conduction of sensory information to the cerebral cortex as well as in the regulation of autonomic functions.

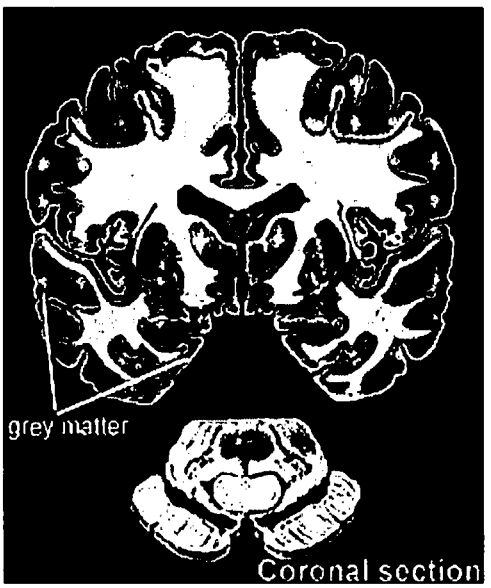

Fig. 2a Normal grey matter
(www.brainexplorer.org)

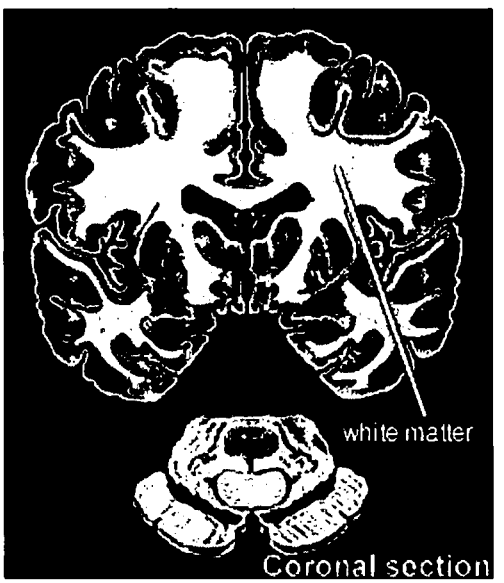

Fig. 2b Normal white matter
(www.brainexplorer.org)

The cerebellum is responsible for psychomotor function while the brainstem forms the link between cerebral cortex, white matter and spinal cord contributing to regulation of breathing, sleeping and circulation.

1.3.1.1 Cells of the CNS:

I. Neurons
II. Glial cells

I. Neurons:

Neurons are cells specialized in the conduction and transmission of electric signals. They are organized into circuits that innervate the body to transmit sensory and motor signals to all areas.

Neurons consists of efferent axons, long nerve-fibers which extend from the cell body and are covered by a myelin sheath. At the end of the axon, the nerve impulses are transmitted to other neurons or effector organs.

Myelin enables nerve impulses to be conducted at a faster rate. A thin myelinated axon transmits impulses at anything from 5 to 30 meters per second whereas an unmyelinated one transmits them at 0.5 to 2 meters per second. It does this both by insulating and containing the nerves. The insulating properties of myelin are due to its structure, the low H_20 content (40%), the thickness and the lipid enrichment.

A nerve impulse is a wave of depolarising current called an action potential that travels along the entire neuron by allowing charged ions of sodium and potassium to flood through channels in the semi-permeable membrane around the nerve cell. At rest (resting potential), the neuron and the surrounding space act as a "capacitor" storing current which is released during the action potential.

Myelin increases the speed of the transmission by containing the current (as positively charged ions) in a small space surrounding the axon. This means that the sodium and potassium ions that contribute to the resting potential do not need to move far when the action potential occurs. Myelin also prevents current from being lost as sodium ions drift away from the neuron.

The myelin sheath is broken at intervals called the nodes of Ranvier which are rich in sodium channels. This makes the nerve impulses move in a stepwise fashion called "salutatory conduction".

Myelin is composed of about 80% lipid fats (cholesterol, phospholipids and glycolipids) and about 20% proteins. Some of the proteins that make up myelin are Myelin Basic Protein (MBP), Myelin Oligodendrocyte Glycoprotein (MOG) and Proteolipid Protein (PLP). Myelin is produced by Oligodendrocytes.

Myelin damage as in MS-patients, results in a severely impaired transmission efficacy.
Dendrites are afferent neuron extensions containing neurofilaments and neurotubuli, typically highly branched and responsible for receiving information and formation of synaptic contacts with the terminals of other nerve cells to allow nerve impuls transmission.

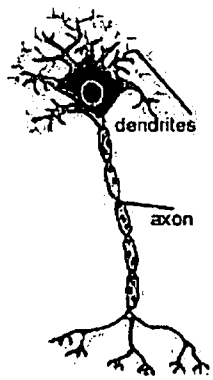

Fig. 3 Neuron
(www.brainexplorer.org)

II. Glial cells

Glial cells are major constituents of the central nervous system.
They can be divided into two groups: microglia and macroglia.
Microglia are phagocytes which are recruited to the CNS by infection or injury.
They do not have a direct role in neurotransmission but microglial cells play a supporting role that helps define synaptic contacts and maintain the signaling abilities of neurons. Their functions are phagocytosis (removal of damaged or

developmental cells), antigen presentation, cytotoxicity and they also act neurotrophic.

Various types of macroglial cells can be found in the brain (or CNS) including astrocytes, oligodendrocytes and Schwann cells. The total number of glial cells exceeds that of neurons by approximately three-fold.

Glial cells are smaller than neurons and lack axons and dendrites. Functions of the glia include: modulating the rate of nerve impulse propagation; controlling the uptake of neurotransmitters; and playing a pivotal role during development and adulthood. Some evidence also suggests that glial cells aid (or, in some cases, prevent) recovery from neuronal injury and that they are involved in a number of diseases, such as Alzheimer's disease, multiple sclerosis and other central and peripheral neuropathies. (D) (E)

Oligodendrocytes (ODCs), formed by Oligodendrocyte Precursor Cells (OPCs), are found in the CNS. Their main function is insulation of axons by forming a myelin sheath and thus increasing nerve impuls velocity. One oligodendrocyte myelinates around 30-50 axons (D) (E).

Schwann cells occur in the peripheral nervous system and do also form myelin sheaths but only envelope one internode of only one axon (D) (E).

Astrocytes represent the highest number of glial cells and responsible for brain homeostastis. Some form end-feet on the surface of neurons in the brain and spinal cord may play a role in bringing nutrients to these cells. Others play end-feet on the brain`s blood vessels and cause the vessel`s endothelial cells to form tight junctions, thus creating the blood-brain barrier (BBB).

They also help in maintaining the right potassium ion concentration in the extracellular space between neurons. They are highly permeable to potassium and can take up an excess of potassium and so protect the neighbouring neurons. An

additional feature is the up-take of neurotransmitters from synaptic zones after release and thereby regulating the synaptic activities.
Astrocytes also provide energy reserves by glycogen storage (D) (E).

1.3.1.2 The Blood-Brain Barrier

The brain is separated from blood only by a very large surface of endothelial cell membranes, the Blood-Brain Barrier (BBB). This barrier maintains a stable environment for neurons by excluding toxic substances. The exclusion results primarily from specialized anatomic properties of brain endothelial cells that limit passive diffusion of water-soluble substances across the vessel walls.
Endothelial cells are interconnected by complex arrays of tight junctions which block diffusion.
Therefore, the BBB is, first of all, a barrier for hydrophobic molecules (proteins, peptides), allowing only the entry of lipophilic substances.
This provides restricted CNS entry of antibodies and inflammatory mediators but also restricted exit of CNS molecules.
Second, the BBB restricts the entry of leucocytes (immune cells) and the exit of CNS derived cells into lymphatic vessels or circulation. An exception are activated T cells and they are able to cross the BBB.
The BBB consists of endothelial cells as a central diffusion barrier, the basement membrane which functions as a molecular filter for size and charge, the membrane glia limitans perivascularis which acts as a ion buffer and allows an active metabolic transport and finally, perivasculare macrophages/microglia cells for phagocytosis and removal of debris (D) (E).

1.3.1.3 Brain Inflammation

The inflammatory process which takes place in the brain is mainly induced and regulated by Class II MHC-restricted T cells.

But inflammation is not only caused by MHC II-restricted $CD4^+$ T_H-1 cells, but also by MHC I-restricted T_c-1 cells as well as by T_H-2/T_c-2 cells.

T_H1-restricted T cells release pro-inflammatory cytokines as INF-γ or TNF-α while T_H2-restricted T cells produce anti-inflammatory cytokines as IL-10, TGF-β. The main effector cells of non antigen-specific bystander damage are tissue-damage microglial cells.

The main elicitor of brain inflammation is the entry of activated T cells across the BBB into the CNS by emperiploesis.

Normally, endothelial cells express a low amount of adhesion molecules and resting T cells have only few binding partners.

During an inflammatory process the expression of adhesion molecules like VCAM, ICAM or LFA-3 is up-regulated by pro-inflammatory cytokines which facilitate the entry of activated T cells into the CNS. These mediators cause tight binding of leucocytes to integrins which results in activation of endothelial cells and stable adhesion of leucocytes. They actively facilitate the entry by dissolving the basal membrane of the BBB.

Inside the CNS chemokines are released which diffuse into the periphery and bind to the surface of endothelial cells, resulting in enhancement of the migratory process.

normal endothelium	*T cell mediated inflammation*
LFA-3	VCAM
PECAM	ICAM
ICAM	PECAM
	P-Selectin
	TNF-α
	chemokines
	neuropeptides
	INF-γ, IL-1β

Tab. 1 Adhesion molecules at the BBB

(source: H. Lassmann: Immunology of neurologic diseases: ftp.hifo.univie.ac.at May, 10th, 2004)

Effector T cells entering the CNS display a migratory phenotype, characterized by the up-regulation of MHC II and the chemokines CCR1, CCR2b, CCR3, CCR5 and CCR7 while Ox 40 and IL-12 are down-regulated. CD4, the TCR and the chemokines CCR4 and CXCR3 remain unchanged.

The expression of MHC molecules in the brain was not discovered until 15 years ago.
Today it is known that the expression of MHC is dependent on the electrical activity, regulated by neurotrophines.
MHC I and MHC II are constitutively expressed by meningeal and perivascular macrophages. MHC I is also expressed by endothelial cells. After stimulation also microglial cells express MHC I and II. During acute inflammation MHC molecules are expressed by all former mentioned cell types as well as by astrocytes.

In summary T cell mediated inflammation on the CNS plays a physiological role by ensuring immune surveillance and tissue degeneration/destruction which allows clearance of debris and supports the regeneration through neurotrophic factors.
The pathologic consequences are either infections like meningitis or the development of autoimmune diseases like multiple sclerosis or Guillane-Barre syndrome.

But autoreactive T cells also might act as a therapeutic target for site-directed drug delivery.

1.3.1.4 CNS Autoimmunity

Autoreactive T cells are a component of the normal immune repertoire.
An autoimmune T cell mediated response against brain proteins may help reducing brain damage, e.g. trauma. Therefore inflammation may act neuroprotective through neurotrophins like NGF, NT3, NT4 or BDNF which are released by inflammatory cells (11;12).
Neuroprotective autoimmunity is mediated by T_H-1 cells and suppressed by $CD4^+/CD25^+$ regulatory T cells (13).
It has shown that there are gender differences displaying a more efficient neuroprotective autoimmunity in females compared to males (14).

1.3.1.5 Mechanisms of inflammatory tissue damage in the CNS

T cell cytotoxicity

Inflammation is characterized by the presence of T cells and activated microglial cells which cause specific damage of antigen containing target cells but can be induced by activated $CD8^+$ cells alone. Without macrophage recruitment no bystander damage is induced. Cytotoxic T cells can cause severe lesions with vasculitis and ischemia.
This is in contrast to CD4+ mediated inflammation where macrophage recruitment leads to massive bystander damage.

T cell mediated inflammation by antibodies

Inflammation in the CNS caused by T cells leads to disruption or disturbances of the Blood-Brain Barrier which leads to an influx of antibodies and complement into the brain. As a result local effector cells are activated.
Antibodies against targets as well as macrophages and sometimes granulocytes, are accessible from the extracellular space and cause selective destruction of antigen-specific target through complement and/or antibody-dependent cellular cytotoxicity (ADCC).

1.3.2 Multiple Sclerosis as a disease

The disease Multiple Sclerosis (MS) was first described by Jean-Marie Charcot (1825-1893) as „la sclerose en plaques desseminées" (F, G, H).
MS is an inflammatory autoimmune disease affecting the Central Nervous System (CNS), i.e. the brain and spinal cord, with an onset in early adulthood – between 20 and 40 years of age and women being affected more often than men (2:1).
The disease is normally not life-shortening but leads to substantial defects in sensation as well as in motor, autonomic and neurocognitive functions.
There are two major forms: Relapsing-Remitting (RR), which is the most frequent form (85-90%) and chronic-progressive. Most of RR patients develop secondary-progressive MS within the years, but about 10-15% develop an insidious onset characterized by steady progression, termed primary-progressive MS.

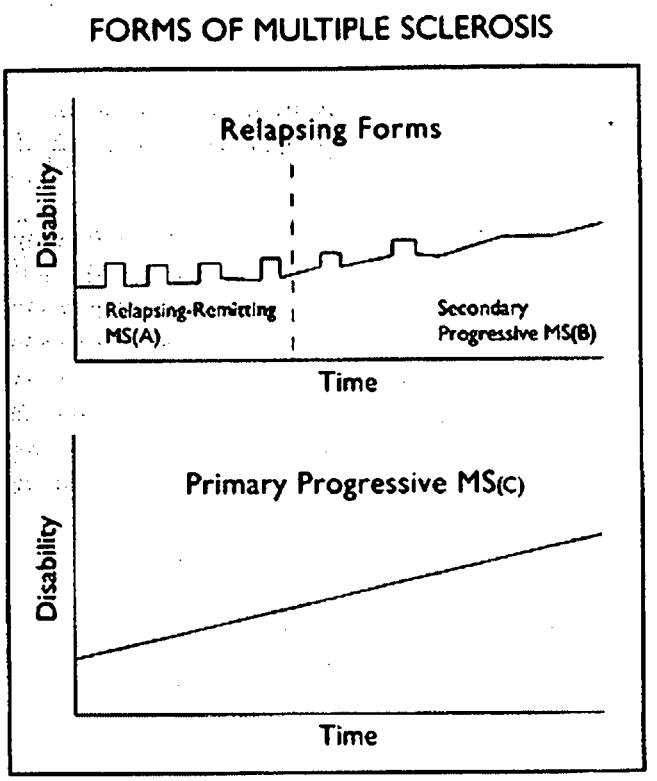

Fig.4 Clinical forms of MS
(http://www.msactivesource.com/msavProject/msas.portal)

MS is characterized by the infiltration of autoreactive T cells into the CNS, localized myelin-destruction, axonal damage and a loss of oligodendrocytes.
Patients have white matter lesions which are detectable by magnetic resonance imaging (MRI).
MS is a heterogenous disease where environmental and genetic factors interact in the pathogenesis. The incidence increases with latitude away from the equator, therefore, areas like northern-Europe or southern Australia being at the highest risk (prevalence varies about factor 10/100.000 persons) (15). A causative factor might be the decrease in sunlight depending on the latitude. UV radiation influences the biosynthesis of vitamin D. Therefore vitamin D receptor polymorphisms have been associated with MS (16;17).
Migration studies have shown that emigration from an area of high prevalence to an area of low prevalence before age 15-16, adapts to the low risk area, whereas migration after that age does not change the risk (18).
That fact might be related to an infectious event aquired during childhood.

The susceptibility to MS varies in different ethnic groups, with people of Caucasian origin displaying the highest disease-rates.
The concordance rate among monozygotic twins is approximately 20-35%, and about 2-5% in first-degree relatives like dizygotic twins and siblings (19;20).

1.3.2.1 Genetic factors

To search for susceptibility genes more than 20 whole genome screens in different MS populations and different geographic areas have been performed e.g. the GAMES study (21), but the results so far are not promising.
HLA-DR2 or-DQ on chromosome 6 is the predominant susceptibility allele associated with MS but even here the frequency varies among patients in different populations (22). Especially in Caucasians the HLA-DR15 haplotype carries a higher risk factor.
There are several other risk-confering genes which have been pointed out as candidate genes for MS, like polymorphisms of CCR2, CCR5, IL-10 receptor α, IL-10, FAS-L, IL-4 receptor α, IL-2 receptor β, INF-γ, NOTCH4 or an allele of apolipoprotein E (APOE4) (19).

Adhesion molecules	**Interleukines/Cytokines**	**Other**
B7-1	*IL-1Ra/IL-1B* (progression)	CD45
CD40L	*IL-2ra/rb/rg* (progression)	Apo B/C2/H
CTLA-4 (progression)	IL-4/R (susceptibility)	*Apo E* (progression)
ICAM-1	Il-7	HLA-DM/DP (susceptibility)
PECAM	Il-9	HLA-DR/DQ (susceptibility)
	IL-10/R	C3/C4
	IL-12 R/p355/p40 (resistence)	TCR A/B (susceptibility)
	INF-α/β	Hsp70
	INF-γ R1/R2	NOS1
	TGF-BR1/BR2/BR3	IgH/V/C
	TNF-α (susceptibility)	FcR (resistence)
	CCR5 (severity)	Rh blood group
	CCL7(MCP-3) resistance	Vitamin D R
	CNTF (severity)	25(OH)D3
		Oestrogen R (severity)
CNS proteins	**Apoptosis**	α-2-macroglobulin
MBP	Apo-1	TIMP-3
PLP	FAS-670 (resistance)	Sca2/3/6/8/11
MOG	p53	Gelatinase B
MAG	Bax, bcl-2, bcl-x	Myeloperoxidase
OMGP		Trk-C
Golli-MBP		GABA A3R
NF2		erb B4
		CYP2D6
		mt DNA

Tab. 2: Candidate genes for MS-susceptibility [Compston, 2003]

1.3.2.2 Non genetic factors

As the concordance rate of monozygotic twins is relatively low, environmental factors are generally suggested to contribute to the etiology of MS.

The higher risk of women to develop MS might be due to the hormonal status. This suggestion is supported by the fact that women during pregnancy have a lower risk for relapses (23).

The *geographical distribution* is also reflected by the economic level of the country and higher prevalence rates are connected with the increasing socioeconomic development (industrialization, urban living, pollution, diet changes, ..), and reduced exposure to infections in developed countries also gave rise to the "hygiene hypothesis" which suggests an increased risk to develop allergies or autoimmune reactions/diseases.

Viral or bacterial infections have long been discussed as candidates for triggering MS. Especially human-pathogenic viruses that induce persistent infections have been investigated, among them Human Herpes Virus 6 (HHV6) or Epstein-Barr Virus (EBV) which have lymphotrophic (HHV6) and neurotrophic properties that cause tissue damage and also have the ability to activate autoimmune responses directed at the myelin tissue. HHV6-DNA has been detected more often in serum and CF of MS-patients than in controls and they exhibit a significantly different cytokine profile (Th1) compared to controls (Th2) (24-27). Some viruses share sequence-homologies with myelin proteins leading to *molecular mimicry*-induced activation of T cells (28). The recognition of self-antigens at intermediate level affinity by T cells during thymic selection results in positive selection and the export of these potentially autoreactive T cells to the periphery where they can crossreact with foreign antigen. The activated T cells now can cross the BBB and infiltrate the CNS. Upon recognition inside the brain tissue damage or the development of autoimmune diseases like MS may be the outcome.

In addition viral CNS infections can also induce autoimmune reactions by epitope spreading and superantigen activity (29).

There are two ways of bystander activation mechanisms to induce autoreactive T cells.

First, TCR-independent bystander activation by inflammatory cytokines, superantigens and molecular pattern recognition, e.g. toll-like receptor (TLR) activation.

During infection chemokines and proinflammatory cytokines are produced which are thought to be the main activators of virus-specific $CD8^+$ cells and inducers of autoimmune processes. Binding of infectious agents to TLRs results in an innate immune response which leads to increased expression of cytokines and reactive oxygen intermediates. For example TLR4 in the CNS is expressed on microglia.

During a bacterial infection TLR4 interacts with LPS which may lead not only to the activation of monocytes and mircroglia but also to the activation of autoreactive T cells in the periphery.

Second, viral tissue damage leads to the unveiling of host antigens.

Here, activated virus-specific T cells travel to the side of infection, recognize viral epitopes and kill the infected cells. The consequence is destruction of self-tissue and release of autoantigens. If these autoantigens are presented together with the adjuvant effect of the infectious agents it will result in de-novo activation of autoreactive T cells and later epitope spreading (15).

1.3.2.3 Main effector cells of MS

Multiple Sclerosis is believed to be a chronic inflammatory autoimmune demyelinating disease of the CNS, which is characterized by patchy inflammatory infiltrates containing blood-derived myelin-specific T cells, B cells secreting anti-myelin component antibodies and various non specific effector mononuclear cells (mostly macrophages).

Current hypothesis revolve around an induction of the autoimmune inflammatory response by T cells in response to one or more infectious agents (molecular mimicry), with the beginning of the disease in the periphery (30).

The first hints that MS is an T cell-derived disorder came from the animal model experimental autoimmune encephalomyelitis (EAE) in the early 1980ies (31;32).

It was observed that EAE could be transferred by in vitro reactivated myelin-specific $CD4^+$ T cells, either adoptive or passive.
EAE can be directly induced by autoreactive T cells in naïve animals, but not by antibodies. This leads to the conclusion that MS is probably a T cell-mediated autoimmune disease (15;33).

$CD4^+$ T cells

CD4+ T cells play a major role in the immunology of the disease.
They are found in the CNS- and CSF-infiltrating inflammatory cells, the genetic risk for MS is partly conferred by HLA-DR and HLA-DQ molecules which code for the T cell related MHCII and antibody production, $CD8^+$ maturation as well as many other innate and adaptive immune reactions are, at least partly, controlled by $CD4^+$.
In the CNS, $CD4^+$ cells target myelin proteins, among them myelin-basic protein (MBP), the best-studied one. There are five isoforms of MBP, according to different splicing sites. The protein is highly basic and positioned at the intracellular surface of myelin membranes in both central and peripheral myelin. It is involved in the maintenance of the myelin structure by forming interactions with acidic lipid moieties.
The most immunodominant epitopes for high-avidity myelin-specific $CD4^+$ T cells are $MBP_{(13-32)}$, $MBP_{(111-129)}$ and $MBP_{(146-170)}$ (34).
The most abundant myelin protein in the CNS is Proteolipid Protein (PLP), a highly hydrophobic and evolutionary conserved protein.
The main targets of PLP-specific high-avidity Th_1 cells are $PLP_{(139-151)}$ and $PLP_{(178-191)}$ (34).
Another target of $CD4^+$ cells in the CNS is Myelin Oligodendrocyte Glycoprotein (MOG), which is located on the outer surface of the oligodendrocyte membrane, which makes it directly accessible to antibodies. Therefore, MOG is a relevant target for both cellular and humoral immune responses. The expression of MOG is less abundant, it is only found in the brain/spinal cord and retina but not in the peripheral lymphoid tissues (35). Immunodominant epitopes are located in the Ig-like extracellular domain as well as in the intracellular parts.

There are several further myelin and non myelin antigens which serve as targets for $CD4^+$ T cells. Among them there are for example *myelin-associated glycoprotein (MAG)* located at the inner surface of the myelin sheath, *2`3`-cyclic nucleotide 3`phosphodiesterase (CNPase)* located in oligodendrocytes, but also expressed by Schwann cells and partly by the lymphoid tissue, *myelin-associated oligodendrocytic basic protein (MOBP)*, an exclusively oligodendrocyte-expressed protein which appears late in myelination, *oligodendrocyte-specific glycoprotein (OSP)*, the third most abundant myelin protein, expressed in the CNS and testis.
The majority of myelin-specific $CD4^+$ T cells are restricted by HLA-DR molecules (15) and display a Th1 phenotype (34).
$CD4^+$ T cells also exhibit cytotoxic activity. They mediate perforin- and Fas/Fas-Ligand-mediated cytotoxicity of MBP. It is considered unlikely that CD4+ cells are directly involved in the lysis of oligodendrocytes or neurons because neither type of CNS cells expresses HLA class II (36;37).

Regulatory $CD4^+CD25^+$ T cells

MS may result from the failure of tolerance mechanisms that prevent the expansion of pathogenic T cells that react against myelin. Tolerance mechanisms include regulatory T cells expressing the transcription factor FoxP3. Studies had shown a decrease in FoxP3 expression in MS patients suggesting impaired immunoregulation by T reg cells (C) (38).
Experiments in animal models have shown that regulatory T cells (Tregs, $CD4^+CD25^+$) are responsible for the prevention of the disease (39).
They suppress T cell proliferation by both cell-cell contact and cytokine-mediated mechanisms. $CD4^+$Th2/3 cells and their cytokines Il-4, Il-10 and TGF-β are therefore thought to be beneficial in MS.
Tregs contribute to the maintenance of peripheral tolerance and breakdown of this tolerance due to neural self-antigens is a main factor in the development of an autoaggressive immune response. Deletion of the $CD4^+CD25^+$ population causes spontaneous autoimmune disease in mice (40).

CD8+ T cells

It is considered that cytotoxic T cells are important effector cells for mediation of pathological immune reaction that induce CNS damage.
$CD8^+$ cells outnumber $CD4^+$ cells in all lesions, not only in active plaques.

	Acute MS % of CD3	Chronic MS % of CD3
Active	67.6% CD8	83.7% CD8
Inactive	52.0% CD8	72.1% CD8

source: H. Lassmann: Immunology of neurologic diseases: ftp.hifo.univie.ac.at May, 10th, 2004

Clonally expanded CD8+ cells (65% compared to 24% $CD4^+$ cells) are found within MS lesions and in the cerebrospinal fluid of MS patients (41).
The TCR repertoire of CD8+ T cells in the CSF resembles the TCR repertoire of brain infiltrating T cells (42). Leukocyte entry into the CNS is tightly regulated by the BBB, but there are at least three different entry routes: from blood to CSF across the choroidal plexus, from blood to subarachnoid space and form blood to parenchymal perivascular space.
MHCI is expressed by oligodendrocytes, astrocytes, axons, neurons and endothelial cells in active lesions, while MHCII is expressed only by microglia cells in the CNS.
That points to the importance of $CD8^+$ cells compared to $CD4^+$ cells.
Some $CD8^+$ T cells can attack neurons and axons directly - by polarizing their cytotoxic granules towards the contact zone – which are expressing MHCI and are therefore susceptible to antigen-specific lysis by cytotoxic T cells (42).
There are also CD8+ virus-specific T cells which are directly capable of lysing neuron via Fas/Fas-L-mediated cytolysis (43).
The CD8+ response to MBP is elevated in MS patients, as there are a number of HLAI – restricted myelin epitopes been described for MBP, PLP, MAG and other proteins (44).
In summary it seems that both T cell populations, $CD4^+$ and $CD8^+$ cells, have an important role in MS.

B cells

The detection of oligoclonal bands in the CSF of MS patients has long been considered as an important clinical parameter in detecting the disease.
Under normal conditions B cells are not able to cross an intact Blood-Brain Barrier.
But under inflammatory conditions B cells, antibodies and complement factors enter the CNS. The activation of B cells can either be due to stimulation with foreign antigen or self antigen through a bystander effect or superantigen stimulation.
There are different ways for B cells to contribute to the pathogenesis of MS.
They can act as antigen-presenting cells (APC) for autoreactive T cells which is underlined by the fact that T cells and B cells often share the same immunodominant epitopes in humans (45).
B cells and tissue bound Ig can also recruit autoreactive T cells into the CNS (46).
Finally, the production of myelin-specific antibodies and the myelin destruction within the MS plaques seem to be the most important way of the contribution of B cells to the pathogenesis of MS.
Antibodies cause demyelination by opsonization of myelin or by complement activation, leading to formation of the membrane-attack complex (MAC) and complement-mediated cytolysis (47).
The most interesting B cell autoantigen in MS is MOG, a target for autoantibody mediated demyelination in experimental autoimmune encephalomyelitis (EAE).
Pathogenic anti-MOG antibodies are mainly directed against conformation-dependent epitopes present on the extracellular immunoglobulin domain of the protein. The autoimmune response might be partly regulated by polymorphisms in the MOG gene itself (48).
The intrathecal IgG response in MS patients also consists of high-affinity anti-DNA antibodies. The mechanisms underlying this triggering of anti-DNA antibodies is still unknown, but may follow the release of large quantities of host DNA from damaged tissue by a primary infection. As under normal conditions DNA is a poor antigen, the production of high-affinity anti-DNA antibodies might be closely related to the autoimmune state in MS (49).

Antibodies may also have a beneficial effect on MS. They can shift the immune response from a TH1-driven to a TH2-driven response (50).
Furthermore, antibodies against CNS components can induce myelin repair.
Intravenous immunoglobulins (IVIG) have also been shown to be effective in autoimmune diseases like MS, as will be discussed below (51;52).

1.3.2.4 MS and innate immune mechanisms

Toll-like receptors (TLR)

The main function of the innate immune system is self-protection and maintenance of homeostasis but under special circumstances it can also result in destructive autoimmunity.
TLRs recognize conserved pathogen-associated molecules and induce proinflammatory signals that induce the adaptive immunity.
They might play a role in breaking peripheral tolerance to self-antigens during chronic infections or inhibit immunosuppressive effects of CD4+CD25+ regulatory T cells on effector T cells via IL-6 by their engagement on dendritic cells (DC) (15).

Mast cells

Mast cell-released mediators (e.g. tryptase and histamine) are increased in the CSF and in acute lesions of MS patients. They act on the disrupted BBB and enhance the entry of leucocytes into the CNS by increased recruitment, adhesion and rolling.
Mast cell proteases such as tryptase and chymase act on the activation of matrix metalloproteinase (MMP) precursors.
In vitro mast cell degranulation in response to MBP leads to demyelination (15).

Nitric Oxide Synthase

Phagocytes (macrophages and granulocytes) are able to generate highly toxic reactive oxygen and nitrogen intermediates which exert strong antimicrobial activities.
Inducible nitric oxide synthase (iNOS) generates nitric oxide (NO), a free radical that is toxic to bacteria. NO, found in MS lesions, can mediate microglia-induced cytotoxicity (53).

Natural Killer Cells (NK)

The association between decreased NK cell activity and MS has been known for over 20 years. Prior and during acute exacerbations NK lysis is reduced which is due to a significantly reduced number of NK cells in general in MS (54).
NK cells could suppress autoimmunity because of their cytokine profile (IL-5, IL-13, TGF-ß) or by target lysis via perforin-and/or TRAIL-dependent mechanisms.
Therefore NK cells may exert important immunoregulatory functions in MS (15).

Complement

Most of the complement found in the CNS is produced by the cells localized in the brain with astrocytes being the major source. The main function of complement is to ensure immune defense against pathogens.
Demyelination results from direct complement activation after complement-binding to myelin and from an autoimmune response against myelin via the classical pathway.
The classical pathway can be activated by purified myelin.
Complement activation, e.g. by MOG, results in oligodendrocyte lysis and chemoattraction of macrophages (15).

Cytokines and Chemokines

Cytokines are proteins that mediate many different responses of innate and adaptive immunity. They are synthesized in response to inflammatory or antigenic stimuli and usually act locally in an autocrine or paracrine fashion by binding to high-affinity receptors on target cells.

For homeostasis a dynamic balance between pro- and anti-inflammatory cytokines is required. Proinflammatory cytokines like INF-γ, TNF-α, IL-12, IL-17, etc. are supposed to play a role in the pathogenesis of MS via activation of the immune system in the periphery and/or by directly damaging the oligodendrocytes or myelin.

Anti-inflammatory cytokines like IL-4 and IL-10 are in contrast considered to be benefical in MS by augmenting B cell proliferation, differentiation and antibody production.

Data on proinflammatory cytokines in MS are contradictory.

Elevated numbers of TNF-α have been reported in blood and serum of MS patients.

But therapy with anti-TNF-α leads to increased exacerbations.

Some data show higher INF-γ levels in MS patients, but a therapeutic trial with INF-γ resulted in exacerbations as well. Also EAE data report different results (15).

Data on anti-inflammatory cytokines are not less divergent.

Especially the role of IL-10 remains unclear.

IL-10 production seems to drop in PBMC, CNS plaques and CSF of MS patients.

It has been reported that IL-10 production is blocked by type I interferons in activated monocytes but stimulated in activated T cells (type I interferons reduce disease exacerbations in early MS). Therefore, it was hypothesized that Il-10 might be differentially regulated in monocytes and T cells (55).

TNF-α belongs to the proinflammatory cytokines but is also involved in tissue repair in the brain. Active demyelinating lesions in the brain of MS patients show elevated levels compared to inactive/remyelinating lesions (56).

Transgenic animal models overexpressing TNF-α and INF-γ induce demyelination, because these cytokines might be toxic for oligodendrocytes. They may activate microglia and macrophages which phagocytose myelin and the proinflammatory

cytokines may be involved in induction of apoptosis with subsequent demyelination (15).
The cystein protease caspase-1 is responsible for processing inflammatory cytokines and is associated with the induction of apoptosis. It might also play a role in the inflammatory and apoptotic processes associated with MS (57).

Chemokines are important for the recruitment of leukocytes and other cells during inflammation. Only disruption of the BBB allows inflammatory cells to enter the CNS.
Trafficking of activated T cells into the brain starts with a weak adhesion and rolling on the endothelial side of the BBB, a firm arrest on the luminal side of the endothelium and finally crossing through the BBB into the CNS. All of these individual steps are induced by chemokines which also form a chemotactic concentration gradient. (source: H. Lassmann: Immunology of neurologic diseases: ftp.hifo.univie.ac.at May, 10th, 2004)

Major receptors on TH1 cells are CCR5 and CXCR3 and on Th2 cells CCR3 and CCR4.
CCR5 might play a pathogenic role in MS, as levels are elevated in circulating T cells. But increased expression of CCR5 was only shown in some studies (58).
T cells of MS patients expressing CCR5 produce high levels of the proinflammatory cytokines INF-γ and TNF-α (59).
A CCR5 Delta32 deletion mutation abolishes functional CCR5 on the cell surface and therefore may reduce the entry of cells into the lesions. But data showed that a lack of CCR5 does not protect from MS but rather predispose to the chronic disease course (60).
The chemokines CCL5 (RANTES) and CXCL10 (IP10) show increased levels in the CSF of MS patients, while CCL2 (MCP-1) is decreased, which correlates with active MRI. This occurs during MS exacerbations but not during remissions and suggests a mainly Th1-driven response in MS (61).
Monocyte chemoattractant protein-1 (MCP-1) plays an important role in many inflammatory and autoimmune diseases and loss of its effector-function alone is sufficient to impair monocyte trafficking in inflammation models. MCP-1 knockout

mice show no clinical and histological EAE disease signs, even if transferred with encephalitogenic T cells. This emphasizes the importance of MCP-1 in the effector phase of the disease (62).
CXCR3 expression may facilitate the entry of T cells into the CSF, while CXCL10 (IP-10) mediates retention in the inflamed brain (15).
EAE data have shown that often the increase of chemokines or their receptors is associated with disease progression while depletion in vivo improves EAE.

1.3.2.5 Pathogenesis of MS

Multiple sclerosis is primarily a demyelinating disease. But acute axonal damage in demyelinating lesions is a frequent event. Demyelination can be partly repaired by mechanisms of remyelination whereas the axonal destruction is irreversible.
The functional impairment in patients with relapsing-remitting MS is mainly caused by inflammation and demyelination in contrast to the accumulations of irreversible neurological deficits which are caused by axonal destruction and loss.
The events leading to demyelination are summarized here:
In the periphery potentially autoreactive T cells are activated, probably by molecular mimicry. Activated T cells, mainly CD4+ cells, can cross the BBB by adhesion to endothelial adhesion molecules (VLA-4, LFA-1) which facilitate their entry into the CNS by transmigration. It is still uncertain if this step involves also a chemokine gradient. Inside the CNS local factors may upregulate the expression of endothelial adhesion molecules which facilitate further entry of autoreactive T cells.
The following brain-inflammation leads to upregulation of proinflammatory cytokines like INF-γ, IL-23, TNF-α or LT as well as of chemokines like RANTES or IL-8. They activate the resident CNS microglia and astrocyte cells, recruit more immune cells (monocytes, mast cells, B cells, $CD8^+$ cells) from the peripheral blood and mediate the formation of inflammatory lesions. The open BBB is a characteristic of acute inflammation and results in tissue edema due to mediator/protease release from mast cells, monocytes and T cells under the

influence of proinflammatory cytokines and oxygen/nitrogen radicals. Several effector mechanisms contribute to myelin damage like direct macrophage-mediated myelin phagocytosis, anti-myelin antibodies secreted by B cells, myelin-toxic cytokines and nitric oxide components. These early inflammatory events already lead to massive CNS (myelin sheath, oligodendrodytes and axons) damage (15).

Recently, inflammation has been questioned to be the exclusive factor leading to demyelination. EAE data showed an increase from 20-80% of morbidity rate in mice treated with an antibody neutralizing INFγ (63). CNS-specific NFγ production can protect mice from progression of disease by inducing a fast clearance of encephalitogenic T cells infiltrating the CNS parenchyma via apoptosis, associated with up-regulation of TNF-Receptor 1 (64).

$CD4^+$ T cells can induce microglia to secrete IL-12 inhibiting factors like PGE_2, thus selflimiting the inflammation (65).

Macrophages may remove myelin debris, therefore, promoting remyelination. They can also induce remyelination by secretion of proinflammatory cytokines (TNF-α) which are able to promote proliferation of oligodendrocyte progenitors via TNFRII signaling (66;67).

Therefore, inflammation in MS is more complex than thought before and includes detrimental and protective components.

Processes leading to myelin damage and axonal loss include direct complement deposition, ADCC via Fc-receptors, antibody-mediated complement activation, myelin phagocytosis, direct axonal lysis by $CD8^+$ cells, secretion of proteases and apoptosis of oligodendrocytes. In addition an increase in glutamate production and a decrease in its degradation leads to glutamate-mediated exocytotoxicity of oligodendrocytes via glutamate-receptor mediated calcium influx (68).

The early phase of MS is in 85% of patients characterized by an acute attack leading to white matter lesions. Axonal loss is predominant in these early appearing lesions and decreases over time. There seems to be a correlation between inflammation and axonal damage as a high amount of damage occurs in areas with large T cell (mainly $CD8^+$) infiltration and the macrophage extent (69).

During the time that follows an inflammatory event demyelinated axons, apoptotic oligodendrocytes and T cells and axonal transsections appear. Astrocytes are activated to proliferate, and macrophages are loaded with phagocytosed myelin

lipids. Th2/Th3 cytokines dominate in lesion resolution and various growth factors, like CNTF and brain-derived neurotrophic factor, are secreted.

Remyelination starts with the activation of oligodendrocyte precursors so that the surviving oligodendrocytes can start repairing the demyelinated areas. Repaired myelin does not possess its former density and therefore conduction velocity is slower.

There is evidence which shows a secondary axonal degeneration, both inside lesions and in the normal appearing white matter (NAWM). Naked, demyelinated axons may be more susceptible to degeneration because of their lost support from oligodendrocytes, a hypothesis supported by the observation of remyelinated axons being protected from further damage (69).

Another assumption is that secondary degeneration might appear due to a decreased remyelination efficacy of oligodendrocytes because of repeated demyelination episodes (70).

Following an inflammatory event the cellular composition of the plaques changes. Chronic plaques show some inflammation but are devoid of inflammatory cells and characterized by myelin loss, axonal damage, an increase in astrocytes and the deposition of scar tissue (15).

Analysis of immunopathological material of actively demyelinated lesions has revealed great heterogeneity in the demyelination patterns among patients.

Four different patterns of pathologic MS have been identified (71).

Pattern I:

Demyelination is macrophage-mediated and lesions are distributed perivenous. Inflammatory infiltrates are composed of T cells and macrophages which are considered, together with microglia, responsible for myelin-degeneration.

The putative mechanisms are T cell-mediated inflammation with macrophage/micorglia activation and demyelination induced by macrophage toxins.

Pattern II:

Demyelination is antibody mediated, and lesions resemble pattern I lesions. In addition they show deposition of immunoglobulins and activated complement at the sites of active destruction. The mechanisms underlying this pattern are T cell-mediated inflammation with macrophage/microglia activation as well as complement-mediated lysis of antibody-targeted myelin.

Pattern III:

Demyelination is due to distal oligodendrogliopathy with inflammation by T cells and macrophages. Small vessel vasculitis with endothelial cell damage and microvessel thrombosis have been seen. Lesions show degeneration of distal oligodendrocyte processes, followed by oligodendrocyte apoptosis and demyelination.

Underlying mechanisms are characterized by T cell-mediated small vessel vasculitis with secondary ischemic damage in the white matter.

Pattern IV:

Similar lesions like in pattern III, but oligodendrocyte degeneration is prominent in a small rim of the white matter. Mechanisms include T cell-mediated inflammation with macrophage/microglia activation. Demyelination is induced by macrophage toxins on the background of metabolically impaired oligodendrocytes. Oligodendrocytes might be genetically defect.

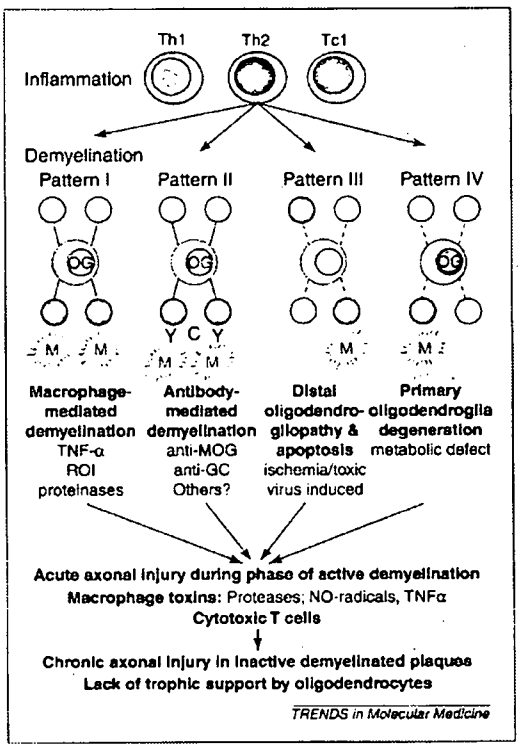

Fig. 5: Heterogenity of MS pathogenesis
(source: Lassmann H., Trends in Molecular Medicine, Vol 7, No.3, March 2001)

Multiple sclerosis is a highly heterogeneous disease in which multiple factors interact in the pathogenesis, and influence the neurological impairment.
First, the number of brain-lesions, their dimension and their location in the CNS.
A lesion at the first relais of the spinal cord effects all other relais, for example.
Surprisingly brain Magnetic Resonance Imaging (MRI) and disability correlate only modest.

Second, the pathology of the lesions and their four different patterns also affect the disease course. Areas with previous inflammation reveal the greatest brain damage.

A new hypothesis proposes that lesion might begin with pattern III and is then followed by an abrupt loss of oligodendrocytes (72).

Normally one patient reveals different lesion types during his/her disease, but it is also possible to find different lesion types at the same time in one patient.

MRI may predict changes in the white matter (WM) almost 18 weeks before lesions appear. Therefore, neurological changes already appear in the preclinical phase of MS.

Third, WM abnormalities affect impairment. Abnormalities are not only seen within lesions, but also in the normal appearing white matter. These are increasingly seen in more disabled patients.

Forth, also the normal appearing grey matter (NAGM) shows damages on MRI of MS patients.

And fifth, the recovery mechanisms, mainly remyelination, play an important role in the pathogenesis of MS. Acute axonal damage is already seen in the early phase of the disease due to inflammation in the brain. The first attack (relapse) can cause severe damage, but may be reversible. Less lesions in the brain mean less brain atrophy. Bone marrow transplantation might stop inflammation, but brain atrophy keeps continuing. The reason are axons, which survive but continue to degenerate which leads to secondary axonal degeneration.

Secondary axonal damage is the predominant factor in the second phase of the disease. While very good results can be achieved by treating patients in the first, acute inflammatory phase with anti-inflammatory drugs, treatment in the second phase shows no results.

Inflammation in the early disease-phase is the key for reversible damage and therefore should be treated (I).

Remyelination

Experimental evidence indicates that surving oligodendrocytes are not the producers of new myelin after a demyelinating event, but oligodendrocyte precursor cells (ODPC) present throughout the adult brain (I).

In response to demyelination these cells proliferate and migrate towards affected areas on neurons to start remyelination of the myelin sheath. This phase is called recruitment phase. For complete remyelination these cells must engage demyelinated axons, differentiate into myelin-sheath forming oligodendrocytes and restore the myelin sheath. This final phase is called differentiation phase.

ODPCs are a normal part of the adult WM and remain mostly quiescent. Only in response to CNS injury they become activated and proliferate. Activation involves an increase in the expression of transcription factor genes, which are associated with developmental myelination. In the NAWM ODPCs express bHLH transcription factor Olig1 but expression of Olig2 and Nkx2.2 is at low level. Following an acute demyelinating event, the expression of both Olig2 and Nkx2.2 dramatically increases. Their expression is confined to the ODPC population. At differentiation of ODPCs into oligodendrocytes and PLP, their expression decreases again. Therefore, it was hypothized that the increased expression of the genes Olig2 and Nkx2.2 in response to demyelination is a critical genetic switch required to convert quiescent ODPCs into cells able to differentiate into remyelinating oligodendrocytes (73).(A)

As there are substantial numbers of premyelinating oligodendrocytes in CNS lesions, the repair-potential seems not to be limited by the loss of these cells. Therefore, interactions between the surrounding environment and oligodendrocytes might be important for the repair process and its success. A micorarray study investigated the links between astrocyte reactivity and lesion repair (74).

During CNS development, contact-mediated activation of Notch1 receptors on ODPCs by the ligand Jagged1 induces Hes5, which in turn inhibits their maturation.

The study found out that TGF-β, which is upregulated in MS patients, specifically reinduced Jagged1 in primary cultures of human astrocytes. Within and around

active plaques, where no remyelination occurs, reactive astrocytes express high levels of Jagged1 and Notch1 and Hes5 were expressed by cells with an immature oligodendrocyte phenotype. TGF-β localized to perivascular extracellular matrix. In contrast to remyelinated areas astrocytes there showed no Jagged1 immunoreactivity. These data suggest that Jagged1-expressing astrocytes may be causally related to the limited remyelination in MS.

Another aspect concerning remyelination is inflammation.

At least some aspects of the inflammatory response associated with demyelination seem to be somehow responsible for subsequent repair processes. This was demonstrated by toxin-induced demyelination models, where the inflammatory response occurs as a consequence of demyelination rather than being the cause of it, like in EAE. Studies on knock-out mice showed an impaired efficiency of remyelination in the absence of proinflammatory cytokines (IL-1 and TNF-α) (67). Thus, inflammatory mediators seem to be necessary for creating a pro-remyelination environment.

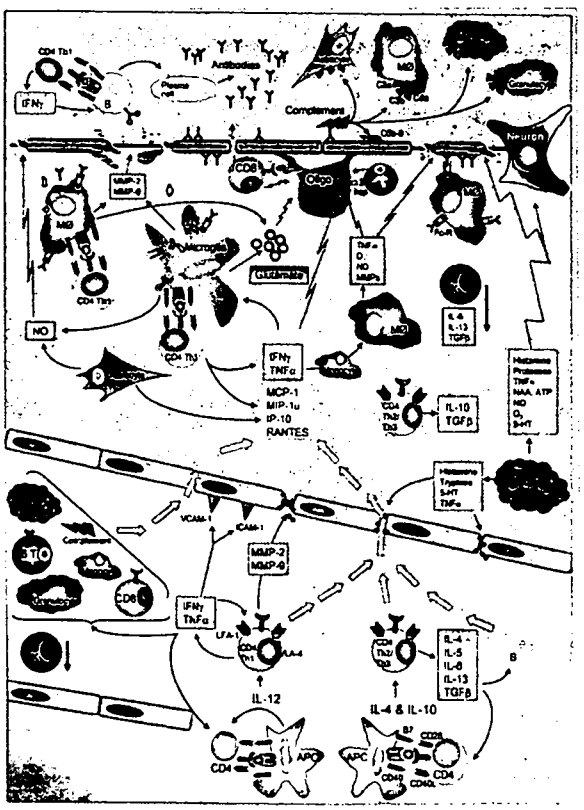

Fig.6 Summary of the pathogenesis of MS
(source: Sospedra M. and Martin R., Annu. Rev. Immunol. 2005, 23: 683-747)

1.3.2.6 Treatment and Therapy

During the last decade several disease-modifying agents have been used as approved therapies for the treatment of relapsing-remitting (RR) MS. Their effectiveness is based on class I evidence – prospective, randomized, controlled clinical trials.
The following drugs are considered as first line therapy:

- interferon beta-1a: Avonex® (Biogen)
- interferon beta-1a: Rebif® (Serono)
- interferon beta-1b: Betaferon® (Schering)
- glatirameracetat: Copaxone® (Teva Pharmaceuticals)

All these drugs have been demonstrated to decrease the relapse rate, slow the progression of disability and improve markers of lesion load observed on MRI.
Concerning disability a study comparing all immunomodulatory drugs did not find a significant reduction in EDSS score (75).
As a second line treatment mitoxandrone (Novandrone® by Serono) - approved only in the USA and natalizumab (Tysabri® by Biogen) are currently used.
For the treatment of inflammatory mediated health-problems, methylprednisolone is widely used.
Intravenous immunoglobulin (IVIG) has shown to be effective in the treatment of MS and will be discussed below in more detail.

Initially, treatment of the disease with modifying drugs was started when "clinically well-established RR disease with high relapse activity" was diagnosed.
Today, due to knowledge on the axonal damage which occurs already in the very early phase of the disease and the percentage of axonal loss, which has a significantly higher percentage than in later stages, there are discussions on whether to start treatment earlier, at signs of the first "clinically isolated syndrome".

Interferons

The benefical effects of interferons in the treatment of MS are due to inhibition of proinflammatory cytokines, induction of anti-inflammatory biological products, reduction of cellular migration and inhibition of autoreactive T cells (76;77).
The INFβ-1b molecule differs from the natural human INFβ by missing a carbohydrate side chain and a serine for cysteine substitution at position 17.
INFβ-1a is glycosylated and basically indistinguishable from natural human INFβ (78).
Experiences have shown that the earlier the treatment is started and the younger the age of the patients, the better the response chances of the interferon therapy.
The EVIDENCE study has shown that a higher dosage and more frequent administration seem to be more effective (79).
A problem of interferon treatment is its immunogenicity. Interferon therapy may induce the appearance of neutralizing antibodies (NABs) already after 6 months of treatment. At the end of a one-year treatment 90% of patients are tested positive for the presence of NABs. The presence of NABs blocks activation of the interferon-receptor and interferon therapy will no longer be effective. Patients tested positive for NABs will also reveal a slightly higher relapse rate compared to patients tested negative for the presence of NABs (80).

Glatirameracetat

It was the third treatment for RR-MS to be approved by the US FDA.
Glatirameracetat (GA) is a standardized, randomized mixture of synthetic polypeptides consisting of L-glutamic acid, L-lysine, L-alanine and L-tyrosine with a defined molecular ratio of 0.14 : 0.34 : 0.43 : 0.09 and an average molecular mass of 4.7 to 11.0kDa, and an average length of 45 to 100 amino acids.
GA has both suppressive and protective effects in EAE and has shown to be effective on clinical and MRI-defined MS patients when daily adiministered s.c.

It is supposed that GA competes in some way with MBP at the antigen-presenting cell level for binding to MHC and GA/MHC competes with MBP/MHC for binding to the TCR. GA induces a shift from a TH1-mediated immune response to a TH2-mediated immune response. The TH2 reactive T cells are able to cross the BBB because of daily immunization. In the CNS they release anti-inflammatory cytokines like IL-4, IL-5, IL-13 and TGF-β which have a benefical effect on the ongoing inflammation in the brain (81).

Mitoxandrone

Patients experiencing relapses while on interferon-therapy are often treated with mitoxandrone, an anthracenedione cytotoxic drug with immunosuppressive properties. Clinical trial showed positive effects for both clinical and MRI-measured endpoints. Mitoxandrone inhibits RNA and DNA synthesis, B cell activity, reduces Th1 activity while enhancing suppressor-T cell activity.
Unfortunately the drug may cause some serious side-effects like cardiotoxicity or even the development of leukemias.

Natalizumab

Natalizumab (Antegren) is a humanized monoclonal antibody against the four subunits of 41-Integrin (VLA-4) and 47-Integrin expressed on leucoytes. The antibody blocks the interaction of the integrins with their ligands VCAM and MadCAM. This causes and inhibition of the transmigration of leucoytes through the BBB.
Clinical trials have shown significant positive effect on the development of new inflammatory lesions in the CNS.
The 2-year AFFIRM study (phase III trial) showed good results in reduction of relapse-rate, reduction of the EDSS score as well as on MRI (data not published yet).
In the SENTINEL study (phase III) natalizumab was compared to placebo in patients using interferon beta-1a where it also showed positive effects concerning relapse-rate and MRI outcomes. (data not published yet).

Natalizumab (Tysabri®) was approved by the FDA in November 2004 but was very recently (February 2005) been taken voluntarily from the market due to severe side-effects of 3 patients in the SENTINEL study (source: www.nationalmssociety.org).

Glucocorticosteroids

Intravenously or orally administered glucocorticosteroids shorten the duration of acute relapses and high dose treatment can delay the development of clinically definite MS for 2 years following the first attack of optic neuritis (78).
Methylprednisolone (MP) shows clinical effects by reducing inflammation and myelin-breakdown as seen on MRI, where the number of gadolinium-enhancing lesions is decreased.
Data indicate that MP suppresses the expression of the adhesion molecules LFA-1, VLA-4 and ICAM-1 on mononuclear cells. Therefore, MP might inhibit the ability, of immune cells to adhere to the endothelium leading to restricted transmigration into the CNS which leads to clinical improvement of patients (82).

New treatments

FTY20 (sphingosine-1-phosphate receptor (S1P) modulator) is an oral immunomodulator capable of reversible sequestering tissue damaging T and B cells away form blood and the CNS to peripheral lymph nodes. The new drug has shown both preventive and therapeutic efficacy in several MS animal models. A proof of concept study demonstrated the effectiveness of both MRI and relapse-related endpoints. It seems that FTY20 has the potential to be an efficious disease modifying treatment for RRMS when administered once daily (A).

Treatment in the progressive phase of MS

Unfortunately disease modifying drugs used for treatment of patients suffering from progressive MS show far less impressive results.
Two large, placebo-controlled studies with interferon beta-1a revealed no effect on sustained disability progression measured by EDSS score though an effect was seen on relapses (I).
Two similar studies using interferon beta-1b came up with divergent results:
The EU-SPMS trial measured a sustained effect on disability while the NA-SPMS trial could not confirm these results. But both studies showed similar positive results concerning the relapse rate and MRI endpoints (I).
Researchers and clinicians assumed that only RRMS was related to inflammation in the brain, while SPMS was thought to be the chronical phase of MS. But evidence shows diffuse WM injuries with severe inflammation in the CNS of SPMS patients. The question why these patients do not respond to immunomodulatory drugs remains open (I).

1.3.3 *Intravenous immunoglobulins and MS*

Intravenous immunoglobulins (IVIG) have been used for the treatment of several autoimmune disorders as immunomodulating agents.
In MS IVIG decreases the relapse-rate and the number of gadolinium-enhancing lesions on MRI. It suppresses the proliferation of activated T cells without modulating the apoptosis rate. Interactions between IVIG and variable regions of autoantibodies seem to be responsible for the ability of IVIG to regulate autoreactive B cell clones in vivo (83). The interaction of IVIG with complement prevents the formation of the C5b-9 membrane attack complex and therefore subsequent tissue damage (83).
EAE-studies indicate that these effects are mediated by modulation of the cytokine network and T cell response. IVIG might also protect oligodendrocytes from phagocytosis mediated by antibodies (84).

The benefical effect of IVIG was shown by a number of open trials (85) and four randomized double-blind studies (52;86-88).

The largest trial, the AIMS study, was performed by Fazekas et al. (85) with 150 patients suffering from RRMS. Patients were treated with either IVIG 0.15-0.20g/kg/month or placebo for a 2-year period. The primary outcome endpoint were changes in EDSS. The patients showed a significant reduction in their EDSS score but the most significant result was a reduction in the mean annual relapse rate. The difference to the placebo group was 59%. Side effects with this low-dose treatment were mild and infrequent.

In the Achiron trial (51) 40 patients participated who were either treated with IVIG 0.4g/kg/day for 5 days followed by 0.4g/kg every 2 month or placebo. The primary endpoint were changes in the annual relapse rate which was achieved in a highly statistically significant way when compared to placebo (from 1.50 to 0.75 in the first year, and to 0.52 in the second year) meaning a reduction of the relapse rate of 63%. T2-weighted MRI images measured semi-quantitatively showed no significant differences.

The study performed by Sorensen (84) on 26 patients suffering from RRMS had new gadolinium-enhancing lesions on MRI as the primary endpoint. Half of the patients were treated with IVIG 2g/kg monthly for 6 months and a 3 months wash-out period after which they were treated with placebo for 6 month. The other half was treated in reverse order. MRI was measured monthly. The outcome was a reduction of ~60% of new lesions in the treatment group. The high-dose IVIG treatment was associated with a high number of side effects.

Lewanska et al. (87) investigated three different IVIG-treatment groups on 49 patients suffering from RRMS. They were treated either with IVIG 0.2g/kg, IVIG 0.4g/kg or placebo monthly over the period of one year. The annual relapse rate decreased significantly, also the EDSS score showed a reduction compared to placebo.

In summary, all four studies confirmed the benefical effect of IVIG on the annual relapse rate of RRMS patients.

Therefore IVIG can be considered as an alternative second-line therapy for the treatment of RRMS (89).

A review summarizing all four trials concluded, that IVIG has indeed beneficial effects on relapses and disability changes in patients suffering from RRMS. The results of all studies, despite the differences in their set-up, were remarkably consistent.

IVIG is a valuable alternative to established therapies for RRMS. Advantages of IVIG treatment include monthly infusions and only mild side-effects if applied in small doses of 0.2-0.4g/kg (85;90).

Nevertheless, the effects of IVIG treatment for RRMS patients is still controversial: as some investigators could show that IVIG is able to enhance CNS remyelination in animal models, a double-blind, placebo controlled trial to evaluate the effect of IVIG treatment in MS patients with a stable clinical deficit was conducted. 10 patients were treated first with placebo and later with IVIG 0.4g/kg for 5 days with a separation periodof 6 weeks between the treatments. The primary outcome parameter was the change in central motor conduction time as an indirect measurement of remyelination. The results of this pilot-study did not support a role for IVIG in the remyelination of patients with stable neurological deficits (91).

Another pilot study, a double-blind, randomized placebo-controlled trial, measured the effectiveness of a combination treatment with IVIG and MP for RRMS patients. As some patients did not show significant improvements of acute relapses after IVMP treatment, the investigators wanted to know if combination therapy with IVIG on top of IVMP would lead to a better efficacy. Patients were randomized for IVMP 500mg directly followed by IVIG 0.4g/kg or placebo for five days. The primary outcome criterion was the EDSS score at four weeks. The results of the study could show no superiority of IVMP-IVIG therapy in the treatment of MS (92).

A recently performed clinical study using MRI as primary outcome measurement for IVIG treatment of patients, suffering from an established clinical neurological deficit caused by RRMS, did not show any significant benefits (93).

Three other small trials studying IVIG treatment at the time of acute relapse of MS patients showed that IVIG did not have any cliniclal influence on the duration of relapses, if given directly after the relapse or optic neuritis. The author of a summarizing review concludes that IVIG is not indicated for the treatment of relapses in MS (94).

Based on the controversial results published in literature, it was the aim of our study to investigate the effects of IVIG treatment in patients suffering from an acute relapse of MS at the level of gene-expression in peripheral T cells, as autoreactive T cells which cross the blood-brain-barrier are the main target of demyelinating antibodies. The aim of my PhD-thesis is the evalutation of differentially expressed genes in peripheral T cells of patients before and after treatment with IVIG.

Initially we intended to use a new method called " subtractive suppression hybridization (SSH)". This method is a PCR-based technique for identifying and isolating cDNAs of differentially expressed genes. It is used to selectively amplify target cDNA fragments which are differentially expressed and simultaneously suppress non target DNA amplification (95).

For further identification of the differentially expressed library created with this method, differential screening must be performed. Finally the identified clones must be confirmed by Northern Blot thus meaning a tedious and lengthy process all together. The initial results that I obtained with this method were not convincing. Furthermore, it was recently suggested that the primary application of this method should be the detection of dramatic alteration in gene expression. The application of this method for the comparison of expression profiles in a set-up where small changes in gene expression are to be expected, was considered to be highly ineffective. Moreover, it was suggested that the target mRNA should be at least 0.1% of the total RNA which means that low abundance genes would not be detected (96). Therfore, we decided to use a more appropriate method and chose a completely different approach – microarray analysis.

Microarray technology has tremendously improved over the last few years. The inclusion of a series of internal controls on the microarray provide a reliable method for gene expression analysis and it was shown by 1064 published articles in the year 2003, the year we started using this method.
(http://www.affymetrix.com/community/publications/full_list.affx?year=2003&result_page=1).

We decided to use the human HU-133 genechip provided by Affymetrix® that allows the detection of ~ 33.000 differentially expressed genes.

2.4 Objectives of my project

Intravenous immunoglobulin (IVIG) have been used successfully in the treatment of several autoimmune disorders, including relapsing-remitting Multiple Sclerosis (RRMS). MS is an inflammatory-mediated disease and a modulation of T cell responses might be involved in the immunomodulatory activities of IVIG.

The aim of my PhD project was to explain the effects of IVIG on gene expression levels. Therefore, I studied the expression profiles of peripheral T cells in patients with RRMS in exacerbation before and after treatment with IVIG using microarrays to identify differentially expressed genes.

My part of the project was the establishment of all cell isolation methods for obtaining total RNA from T cells obtained from peripheral blood mononuclear cells (PBMC), isolation of RNA, data analysis of microarrays and confirmation of microarray results using Real Time PCR.

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1.3.2.6 Treatment and Therapy

During the last decade several disease-modifying agents have been used as approved therapies for the treatment of relapsing-remitting (RR) MS. Their effectiveness is based on class I evidence – prospective, randomized, controlled clinical trials.
The following drugs are considered as first line therapy:

- interferon beta-1a: Avonex® (Biogen)
- interferon beta-1a: Rebif® (Serono)
- interferon beta-1b: Betaferon® (Schering)
- glatirameracetat: Copaxone® (Teva Pharmaceuticals)

All these drugs have been demonstrated to decrease the relapse rate, slow the progression of disability and improve markers of lesion load observed on MRI.
Concerning disability a study comparing all immunomodulatory drugs did not find a significant reduction in EDSS score (75).
As a second line treatment mitoxandrone (Novandrone® by Serono) - approved only in the USA and natalizumab (Tysabri® by Biogen) are currently used.
For the treatment of inflammatory mediated health-problems, methylprednisolone is widely used.
Intravenous immunoglobulin (IVIG) has shown to be effective in the treatment of MS and will be discussed below in more detail.

Initially, treatment of the disease with modifying drugs was started when "clinically well-established RR disease with high relapse activity" was diagnosed.
Today, due to knowledge on the axonal damage which occurs already in the very early phase of the disease and the percentage of axonal loss, which has a significantly higher percentage than in later stages, there are discussions on whether to start treatment earlier, at signs of the first "clinically isolated syndrome".

Interferons

The benefical effects of interferons in the treatment of MS are due to inhibition of proinflammatory cytokines, induction of anti-inflammatory biological products, reduction of cellular migration and inhibition of autoreactive T cells (76;77).
The INFβ-1b molecule differs from the natural human INFβ by missing a carbohydrate side chain and a serine for cysteine substitution at position 17.
INFβ-1a is glycosylated and basically indistinguishable from natural human INFβ (78).
Experiences have shown that the earlier the treatment is started and the younger the age of the patients, the better the response chances of the interferon therapy.
The EVIDENCE study has shown that a higher dosage and more frequent administration seem to be more effective (79).
A problem of interferon treatment is its immunogenicity. Interferon therapy may induce the appearance of neutralizing antibodies (NABs) already after 6 months of treatment. At the end of a one-year treatment 90% of patients are tested positive for the presence of NABs. The presence of NABs blocks activation of the interferon-receptor and interferon therapy will no longer be effective. Patients tested positive for NABs will also reveal a slightly higher relapse rate compared to patients tested negative for the presence of NABs (80).

Glatirameracetat

It was the third treatment for RR-MS to be approved by the US FDA.
Glatirameracetat (GA) is a standardized, randomized mixture of synthetic polypeptides consisting of L-glutamic acid, L-lysine, L-alanine and L-tyrosine with a defined molecular ratio of 0.14 : 0.34 : 0.43 : 0.09 and an average molecular mass of 4.7 to 11.0kDa, and an average length of 45 to 100 amino acids.
GA has both suppressive and protective effects in EAE and has shown to be effective on clinical and MRI-defined MS patients when daily adiministered s.c.

It is supposed that GA competes in some way with MBP at the antigen-presenting cell level for binding to MHC and GA/MHC competes with MBP/MHC for binding to the TCR. GA induces a shift from a TH1-mediated immune response to a TH2-mediated immune response. The TH2 reactive T cells are able to cross the BBB because of daily immunization. In the CNS they release anti-inflammatory cytokines like IL-4, IL-5, IL-13 and TGF-β which have a benefical effect on the ongoing inflammation in the brain (81).

Mitoxandrone

Patients experiencing relapses while on interferon-therapy are often treated with mitoxandrone, an anthracenedione cytotoxic drug with immunosuppressive properties. Clinical trial showed positive effects for both clinical and MRI-measured endpoints. Mitoxandrone inhibits RNA and DNA synthesis, B cell activity, reduces Th1 activity while enhancing suppressor-T cell activity.
Unfortunately the drug may cause some serious side-effects like cardiotoxicity or even the development of leukemias.

Natalizumab

Natalizumab (Antegren) is a humanized monoclonal antibody against the four subunits of 41-Integrin (VLA-4) and 47-Integrin expressed on leucoytes. The antibody blocks the interaction of the integrins with their ligands VCAM and MadCAM. This causes and inhibition of the transmigration of leucoytes through the BBB.
Clinical trials have shown significant positive effect on the development of new inflammatory lesions in the CNS.
The 2-year AFFIRM study (phase III trial) showed good results in reduction of relapse-rate, reduction of the EDSS score as well as on MRI (data not published yet).
In the SENTINEL study (phase III) natalizumab was compared to placebo in patients using interferon beta-1a where it also showed positive effects concerning relapse-rate and MRI outcomes. (data not published yet).

Natalizumab (Tysabri®) was approved by the FDA in November 2004 but was very recently (February 2005) been taken voluntarily from the market due to severe side-effects of 3 patients in the SENTINEL study (source: www.nationalmssociety.org).

Glucocorticosteroids

Intravenously or orally administered glucocorticosteroids shorten the duration of acute relapses and high dose treatment can delay the development of clinically definite MS for 2 years following the first attack of optic neuritis (78).
Methylprednisolone (MP) shows clinical effects by reducing inflammation and myelin-breakdown as seen on MRI, where the number of gadolinium-enhancing lesions is decreased.
Data indicate that MP suppresses the expression of the adhesion molecules LFA-1, VLA-4 and ICAM-1 on mononuclear cells. Therefore, MP might inhibit the ability, of immune cells to adhere to the endothelium leading to restricted transmigration into the CNS which leads to clinical improvement of patients (82).

New treatments

FTY20 (sphingosine-1-phosphate receptor (S1P) modulator) is an oral immunomodulator capable of reversible sequestering tissue damaging T and B cells away form blood and the CNS to peripheral lymph nodes. The new drug has shown both preventive and therapeutic efficacy in several MS animal models. A proof of concept study demonstrated the effectiveness of both MRI and relapse-related endpoints. It seems that FTY20 has the potential to be an efficious disease modifying treatment for RRMS when administered once daily (A).

Treatment in the progressive phase of MS

Unfortunately disease modifying drugs used for treatment of patients suffering from progressive MS show far less impressive results.
Two large, placebo-controlled studies with interferon beta-1a revealed no effect on sustained disability progression measured by EDSS score though an effect was seen on relapses (I).
Two similar studies using interferon beta-1b came up with divergent results:
The EU-SPMS trial measured a sustained effect on disability while the NA-SPMS trial could not confirm these results. But both studies showed similar positive results concerning the relapse rate and MRI endpoints (I).
Researchers and clinicians assumed that only RRMS was related to inflammation in the brain, while SPMS was thought to be the chronical phase of MS. But evidence shows diffuse WM injuries with severe inflammation in the CNS of SPMS patients. The question why these patients do not respond to immunomodulatory drugs remains open (I).

1.3.3 *Intravenous immunoglobulins and MS*

Intravenous immunoglobulins (IVIG) have been used for the treatment of several autoimmune disorders as immunomodulating agents.
In MS IVIG decreases the relapse-rate and the number of gadolinium-enhancing lesions on MRI. It suppresses the proliferation of activated T cells without modulating the apoptosis rate. Interactions between IVIG and variable regions of autoantibodies seem to be responsible for the ability of IVIG to regulate autoreactive B cell clones in vivo (83). The interaction of IVIG with complement prevents the formation of the C5b-9 membrane attack complex and therefore subsequent tissue damage (83).
EAE-studies indicate that these effects are mediated by modulation of the cytokine network and T cell response. IVIG might also protect oligodendrocytes from phagocytosis mediated by antibodies (84).

The benefical effect of IVIG was shown by a number of open trials (85) and four randomized double-blind studies (52;86-88).

The largest trial, the AIMS study, was performed by Fazekas et al. (85) with 150 patients suffering from RRMS. Patients were treated with either IVIG 0.15-0.20g/kg/month or placebo for a 2-year period. The primary outcome endpoint were changes in EDSS. The patients showed a significant reduction in their EDSS score but the most significant result was a reduction in the mean annual relapse rate. The difference to the placebo group was 59%. Side effects with this low-dose treatment were mild and infrequent.

In the Achiron trial (51) 40 patients participated who were either treated with IVIG 0.4g/kg/day for 5 days followed by 0.4g/kg every 2 month or placebo. The primary endpoint were changes in the annual relapse rate which was achieved in a highly statistically significant way when compared to placebo (from 1.50 to 0.75 in the first year, and to 0.52 in the second year) meaning a reduction of the relapse rate of 63%. T2-weighted MRI images measured semi-quantitatively showed no significant differences.

The study performed by Sorensen (84) on 26 patients suffering from RRMS had new gadolinium-enhancing lesions on MRI as the primary endpoint. Half of the patients were treated with IVIG 2g/kg monthly for 6 months and a 3 months wash-out period after which they were treated with placebo for 6 month. The other half was treated in reverse order. MRI was measured monthly. The outcome was a reduction of ~60% of new lesions in the treatment group. The high-dose IVIG treatment was associated with a high number of side effects.

Lewanska et al. (87) investigated three different IVIG-treatment groups on 49 patients suffering from RRMS. They were treated either with IVIG 0.2g/kg, IVIG 0.4g/kg or placebo monthly over the period of one year. The annual relapse rate decreased significantly, also the EDSS score showed a reduction compared to placebo.

In summary, all four studies confirmed the benefical effect of IVIG on the annual relapse rate of RRMS patients.

Therefore IVIG can be considered as an alternative second-line therapy for the treatment of RRMS (89).

A review summarizing all four trials concluded, that IVIG has indeed beneficial effects on relapses and disability changes in patients suffering from RRMS. The results of all studies, despite the differences in their set-up, were remarkably consistent.

IVIG is a valuable alternative to established therapies for RRMS. Advantages of IVIG treatment include monthly infusions and only mild side-effects if applied in small doses of 0.2-0.4g/kg (85;90).

Nevertheless, the effects of IVIG treatment for RRMS patients is still controversial: as some investigators could show that IVIG is able to enhance CNS remyelination in animal models, a double-blind, placebo controlled trial to evaluate the effect of IVIG treatment in MS patients with a stable clinical deficit was conducted. 10 patients were treated first with placebo and later with IVIG 0.4g/kg for 5 days with a separation periodof 6 weeks between the treatments. The primary outcome parameter was the change in central motor conduction time as an indirect measurement of remyelination. The results of this pilot-study did not support a role for IVIG in the remyelination of patients with stable neurological deficits (91).

Another pilot study, a double-blind, randomized placebo-controlled trial, measured the effectiveness of a combination treatment with IVIG and MP for RRMS patients. As some patients did not show significant improvements of acute relapses after IVMP treatment, the investigators wanted to know if combination therapy with IVIG on top of IVMP would lead to a better efficacy. Patients were randomized for IVMP 500mg directly followed by IVIG 0.4g/kg or placebo for five days. The primary outcome criterion was the EDSS score at four weeks. The results of the study could show no superiority of IVMP-IVIG therapy in the treatment of MS (92).

A recently performed clinical study using MRI as primary outcome measurement for IVIG treatment of patients, suffering from an established clinical neurological deficit caused by RRMS, did not show any significant benefits (93).

Three other small trials studying IVIG treatment at the time of acute relapse of MS patients showed that IVIG did not have any cliniclal influence on the duration of relapses, if given directly after the relapse or optic neuritis. The author of a summarizing review concludes that IVIG is not indicated for the treatment of relapses in MS (94).

Based on the controversial results published in literature, it was the aim of our study to investigate the effects of IVIG treatment in patients suffering from an acute relapse of MS at the level of gene-expression in peripheral T cells, as autoreactive T cells which cross the blood-brain-barrier are the main target of demyelinating antibodies. The aim of my PhD-thesis is the evalutation of differentially expressed genes in peripheral T cells of patients before and after treatment with IVIG.

Initially we intended to use a new method called " subtractive suppression hybridization (SSH)". This method is a PCR-based technique for identifying and isolating cDNAs of differentially expressed genes. It is used to selectively amplify target cDNA fragments which are differentially expressed and simultaneously suppress non target DNA amplification (95).

For further identification of the differentially expressed library created with this method, differential screening must be performed. Finally the identified clones must be confirmed by Northern Blot thus meaning a tedious and lengthy process all together. The initial results that I obtained with this method were not convincing. Furthermore, it was recently suggested that the primary application of this method should be the detection of dramatic alteration in gene expression. The application of this method for the comparison of expression profiles in a set-up where small changes in gene expression are to be expected, was considered to be highly ineffective. Moreover, it was suggested that the target mRNA should be at least 0.1% of the total RNA which means that low abundance genes would not be detected (96). Therfore, we decided to use a more appropriate method and chose a completely different approach – microarray analysis.

Microarray technology has tremendously improved over the last few years. The inclusion of a series of internal controls on the microarray provide a reliable method for gene expression analysis and it was shown by 1064 published articles in the year 2003, the year we started using this method.
(http://www.affymetrix.com/community/publications/full_list.affx?year=2003&result_page=1).

We decided to use the human HU-133 genechip provided by Affymetrix® that allows the detection of ~ 33.000 differentially expressed genes.

2.4 Objectives of my project

Intravenous immunoglobulin (IVIG) have been used successfully in the treatment of several autoimmune disorders, including relapsing-remitting Multiple Sclerosis (RRMS). MS is an inflammatory-mediated disease and a modulation of T cell responses might be involved in the immunomodulatory activities of IVIG.

The aim of my PhD project was to explain the effects of IVIG on gene expression levels. Therefore, I studied the expression profiles of peripheral T cells in patients with RRMS in exacerbation before and after treatment with IVIG using microarrays to identify differentially expressed genes.

My part of the project was the establishment of all cell isolation methods for obtaining total RNA from T cells obtained from peripheral blood mononuclear cells (PBMC), isolation of RNA, data analysis of microarrays and confirmation of microarray results using Real Time PCR.

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3.1 Abstract

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of autoimmune diseases of the central nervous system including multiple sclerosis (MS).

Although IVIG seems to have a substantial effect on short- and long-term treatment potential, the underlying mechanisms of action are not elucidated.

It has been suggested that a modulation of the T cell immune responses might be involved in the benefical effects of IVIG therapy and therefore we focused on the identification and characterization of genes involved in the immunomodulatory activity of IVIG in the treatment of exacerbations in Relapsing-Remitting MS (RRMS).

Using microarrays we investigated the expression profiles of T cell fractions of peripheral blood mononuclear cells (PBMC) isolated from 10 RRMS patients treated with IVIG as well as five control patients treated with intravenous methylprednisolone (IVMP). Among the approximately 33.000 genes examined, we found 152 different genes (176 probe-sets) which were differentially regulated by a minimum of a two-fold change in at least 40% of patients. They included genes involved in immune response, inflammatory response, proliferation, apoptosis, cell cycle, signal transduction or regulation of transcription. Statistical analysis by parametric t-test revealed a different number of significantly differentially regulated genes. When comparing the results obtained with both approaches, only a few genes were in common. Differences in the results obtained from gene expression profiles using different approaches for the evaluation of the data are a known problem in the literature. International standartization of statistical approaches for the evaluation of gene expression data obtained from microarray analysis will be necessary to generate reproducible and comparable results in different laboratories.

3.2 Introduction

Multiple Sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS). It is characterized by the infiltration of immune cells, mainly activated T cells, into the brain accompanied by a disruption of the blood-brain-barrier (BBB). In the white matter of the CNS demyelinating lesions lead to neurological deficits.

Intravenous immunoglobulins (IVIG) have been shown to be effective in the treatment of a number of autoimmune diseases including MS (1).

But the precise mechanism of action underlying the immunomodulatory activities of IVIG has not been elucidated. There are several models to explain the immunomodulatory potential of IVIG in patients suffering from autoimmune and inflammatory diseases, including Fcγ-receptor-mediated immunomodulation, influence on idiotype/anti-idiotype network, elimination of immunostimulating microbial products or neutralizing antibodies against cytokines and chemokines.

A potential to modify the balance of TH1 and TH2 cells and an inhibition of the formation of antibody/complement complexes have also been shown (2).

In MS IVIG decreases the relapse-rate and the number of gadolinium-enhancing lesions on brain-MRI. Furthermore, it suppresses the proliferation of activated T cells without modulating the apoptosis rate (3-6).

The benefical effect of IVIG in MS was shown by a number of open trials (3) and four randomized double-blind studies (5;7-9).

It was the aim of our study to investigate the effects of IVIG treatment in patients suffering from an acute relapse of MS at the level of gene-expression in peripheral T cells, as auto-reactive T cells can cross the blood-brain-barrier and are the main effector cells causing brain inflammation.

To investigate the mechanism of action of IVIG, a small clinical study including 10 patients suffering from RRMS was performed. Patients were treated with IVIG for 5 days and magnetic-resonsance imaging (MRI) - for measurement of treatment efficacy - was taken before treatment, 1 day after completion of therapy on day 6 as well as 3 weeks after termination of therapy on day 21. A group of five patients treated with methylprednisolone (IVMP) were used as a control.

Microarray technology has significantly improved over the last few years and allows a systematical analysis of the expression of a great number of genes. Especially Affymetrix technology has shown high correlation among replicates and low levels of variance when compared to other microarray platforms (10).

Analysis of microrarrays applying a filter criteria of a 2-fold change of expression revealed 152 differentially regulated genes and a number of 176 different probe-sets in at least 40% of the patients, including 57 genes involved in regulation of immune responses or inflammatory response.

Statistical analysis of the same data set using a parametric t-test detected 360 different probe-sets. These results show that data-analysis is not unique and discrepancies are possible for data-sets of human diseases (11). The results of two data-sets were confirmed by Real Time PCR.

The efficacy of IVIG treatment was also confirmed by the clinical results, which showed a reduction in the Enhanced Disability Status Scale (EDSS) of MS patients after IVIG treatment.

3.3 Materials and Methods

3.3.1 Patients involved in the study

All patients included in the study were suffering from clinically and laboratory-supported definite MS of Relapsing-Remitting type. The diagnosis of MS was based on Poser`s criteria. The neurological disability was evaluated by the Expanded Disability Status Scale (EDSS). Patients characteristics are listed in table 1. Before entry into the study each patient signed a form of consent to participate in the study. The study was approved by the Ethical Committee of Tampere University, Tampere, Finnland.

Patients who received prior treatment with immunosuppressants in the preceding nine months or patients who received corticosteroids in the preceding eight weeks were excluded.

All patients received Endobulin® (Baxter AG, Vienna, Austria) in a 5-day course of 0.4g/kg/day.

Clinical evaluation of the patients was done before treatment with IVIG, 1 day after completion of therapy on day 6 as well as 3 weeks after completion of therapy on day 21. It included neurological examination, determination of the EDSS score, arm index and Ambulation index.

The primary outcome of the study was changes in the EDSS score from baseline (before IVIG) to week 3.

Secondary outcome measurement points were changes in the volume or number of several MRI-measures (T1-, T2-, FLAIR- and Gadolinium-enhancing lesions as well as brain volume).

A control-group of 5 patients was treated with 1g/day of intravenous methylprednisolone (IVMP) for 3 days what represents the current standard of care.

Table. 1

Characteristics of study population

Characteristics	IVIG Patients	IVMP Controls
Number of study population	10	5
Age average ± SD, year)	40 ± 10.6	35.3 ± 8.8
Sex (male vs female)	3 vs 7	0 vs 5
Disease duration (average ± SD, year)	5.6 ± 3.5	5.2 ± 3.6
Time current vs previous relapse (average ± SD, month)	17.6 ± 21.0	5 ± 3.2
EDSS score at remission (average ± SD, score)	3.7 ± 1.1	3.2 ± 2.4
EDSS score at acute relapse (average ± SD, score)	3.7 ± 1.1	4.2 ± 2.0

A total of 10 patients suffering from Multiple Sclerosis in acute relapse were selected for a clinical trial to confirm the efficacy of a 5-day course of IVIG-treatment on EDSS score and brain-MRI to improve disease-condition.
A total of 5 patients RRMS patients were treated with a 3-day course of IVMP as a control.

3.3.2 Sample Preparation

Peripheral blood mononuclear cells (PBMC) were separated from peripheral blood in Vacutainer® CPTTM Cell Preparation Tubes (Becton Dickinson, Franklin Lakes, N.J.) within 60 min after blood sampling using standard procedures of density gradient (Lymphoprep, Nycomed, Roskilde, DK) centrifugation.

PBMCs were separated into T cells and non T cells using a mixture of non-stimulating anti-$CD4^+$ and anti-$CD8^+$ magnetic Dynabeads (Dynal Biotech, Oslo, N) at 4°C.

Aliquots of ~ $5x10^6$ cells were pelleted and the pellets were thoroughly mixed with 1ml TRIzol (Invitrogen, Carlsbad, CA). Aliquots were stored and frozen at –80° until further processing.

Total RNA was isolated according to the manufacturer's protocol and RNA pellets were dissolved in Nuclease-free water (Invitrogen, Carlsbad, CA), measured and stored at –80°.

3.3.3 Microarrays

For this study we used the HU-133A Genechip (Affymetrix, Santa Clara, CA) containing approximately 33.000 genes. 5µg of total T cell RNA were in-vitro transcribed, labelled and hybridized on the array according to the manufacturer's protocol (see Affymetrix.com) RNA quality was checked before in-vitro processing with a Bioanalyzer (Agilent Technologies, Palo Alto, CA).

3.3.4 Data Analysis

Data were analyzed with Gene chip Operating software (GCOS) and Data Mining Tool software (DMT) (Affymetrix, Santa Clara, CA). We searched for genes which were either up- or down-regulated in 40%, 50%, 60%, 80% and 100% of patients. Cut-off values were a 2-fold differential expression with a p-value of $\leqq 0.006$ or $\geqslant 0.994$.

3.3.5 Real Time Polymerase chain reaction

The microarray data for 8 genes were confirmed by quantitative real-time PCR.
1µg of total T cell RNA was used for reverse transcription into cDNA according to the manufacturer's protocol (MBI Fermentas, Burlington, Canada).
100ng cDNA in 5µl nuclease-free water (Invitrogen, Carlsbad, CA) were quantitatively analyzed using different TaqMan® Assays-on-Demand (Applied Biosystems, Foster City, CA) with the ABPrism 7000 (Applied Biosystems).
Data analysis was performed using the ^^CT-Method, a method commonly used for relative quantification (12). For normalization of expression data human GPDH

was used as a housekeeping gene. For verification of normalization, a second housekeeping gene, β-2microglobulin, was used as a control (data not shown).

3.3.6 Statistical Analysis

Our data set of 30 arrays (3 time points /10 replicates each) was analyzed using a parametric t-test (Welch t-test with unpaired variances). First, all arrays were normalized followed by normalization of genes and logarithmic transformation of all data. Genes with a fold change ≥ 2 as well as a p-value <0.5 were significantly changed. The same method was used for analyzing the control group with a lower-sample number (5 patients).

3.4 Results

3.4.1 Clinical outcome

The clinical outcome of the study showed that a 5-day course of IVIG therapy of patients resulted in a significant reduction of the EDSS score in all 10 patients from a mean of 3.8 to 2.6 (Fig. 1, Tab.2) as well as in an improvement of MRI measurements (Tab. 3a), for example from a median of 1.76 down to 1.73 on T1-weighted images (used for the detection of axonal loss) or from a median of 5.49 down to 5.08 on T2-weighted images (used for the detection of new lesion in the brain). Data for MRI-measurements of the control group are given in Tab. 3b. Here, IVMP does not have an overall reducing effect on MRI measurements as seen on the median for the T1-weighted images.
Treatment was save and well-tolerated. Based on these data, we could confirm the benefical effects of IVIG in patients with RRMS during relapse.

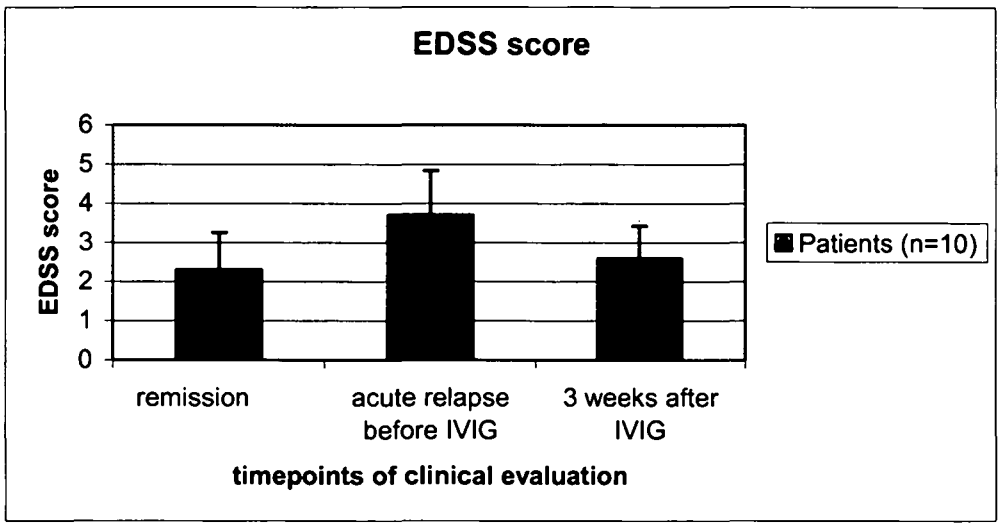


Figure. 1 Treatment effect of IVIG on mean EDSS score of patients

Table. 2 Treatment efficacy of IVIG on mean EDSS score of patients

Names	*Group*	*EDSS at day 0*	*EDSS at day 6*	*EDSS at day 21*
NK	IVIG	2	4	3
MP	IVIG	0.5	2	1
AH	IVIG	4	5	4
PJ	IVIG	2	3	2.5
MN	IVIG	2.5	3	2.5
LM	IVIG	3	4	3
TV	IVIG	2.5	3	2.5
IL	IVIG	3	5	2
AT	IVIG	2.5	3	2.5
PL	IVIG	3	5.5	4
HA	IVIG	1.5	3.5	1.5
LT	IVIG	3	5	3

Table 3a

MRI measurements of brain abnormalities before and after IVIG

Parameter	Before IVIG	After IVIG
Lesion vol cm3	mean ± SE	mean ± SE
T1	1. 76 ± 0.55	1.73 ± 0.59
T2	5.49 ± 1.09	5.08 ± 1.03*
Flair	15.76 ± 2.23	14.09 ± 1.94**
Gd-enhanced	0.32 ± 0.27	0.21 ± 0.24**
Brain volume	1124.94 ± 40.61	1120.31± 40.72
Gd+lesion N	2.83 ± 0.71	2.00 ± 0.60**
EDSS° score	3.8 ± 0.3	2.6 ± 0.2**

* p<0.05; ** p<0.01
° EDSS = Kurtzke`s Expanded Disability Status Scale

Table 3b:

MRI measurements of brain abnormalities before and after IVMP

Parameter	Before IVMP	After IVMP	P value
Lesion vol cm3	mean ± SE	mean ± SE	
T1	1.41 ± 0.60	1.64 ± 0.84	1
T2	11.15 ± 4.59	9.83 ± 4.17	0.17
Flair	24.37 ± 8.19	23.18 ± 8.05	0.25
Gd-enhanced	0.70 ± 0.39	0.63 ± 0.37	0.59
Brain volume	1056.32 ± 47.78	1045.07 ± 52.53	0.35
Gd+lesion N	3.0 ± 1.5	2.7 ± 1.4	0.16
EDSS* score	Not available	Not available	

*EDSS = Kurtzke`s Expanded Disability Status Scale

3.4.2 Gene expression profiles

Microarray analysis of gene expression analysis examined ~22.000 transcripts and resulted in 176 differentially expressed probe-sets and 152 differentially expressed genes after application of a 2-fold criteria and a concomitance of a minimum of 40% in all patients after treatment with IVIG (Tab. 4). The majority of probe-sets (134) were down-regulated between day 6 and day 21 after beginning of IVIG therapy, while the only up-regulations (29) occurred at day 6 compared to day 0 after start of treatment (Tab. 5). A complete list of genes is given in the attachment section (see table A1).

Out of these genes, 49 probe-sets are involved in either immune response or inflammatory response (see Tab. A1). An example of two immune-relevant genes which were common in 60% of patients (6 out of 10) were the Fc fragment of IgG receptor 1 - FCGR1 (CD64) - and the Fc fragment of IgG receptor 2A - FCGR2A (CD32). Both genes were down-regulated at day 21 compared to day 6, while CD64 was up-regulated at day 6 compared to day 0 as well.

FCGR1 (CD64) is the only high affinity receptor in the immunoglobulin receptor superfamily due to its third extracellular domain and has a specifity for IgG1 and IgG3. It can bind monomeric and aggregated Ig and functions mainly in phagocytosis. FCGR2 (CD32) is a low affinity receptor which binds IgG in form of immune complexes with a specificity for IgG1 and IgG3. FCGR2A is mainly expressed by macrophages and neutrophils and functions in phagocytosis and cell activation but it is also expressed by a small subset of activated T cells where it fuctions as a cytolytic receptor in ADCC (Antibody-dependent cell-mediated cytotoxicity).

Probe-sets not involved in immune or inflammatory response reveal their main function in signalling, transcription, regulation of proliferation or cell cycle.

Microarray analysis of gene expression of the control-group showed 784 differentially regulated prob-sets after application of a 2-fold criteria and a concomitance of a minimum of 40% in all patients after treatment with IVMP (Tab. 6). Again, the majority of genes showing a change in expression was down-regulated. The highest number of down-regulation occurred at day 6 compared to day 0 after beginning of IVMP treatment (Tab. 7).

Out of the list containing probe-sets differentially regulated upon IVIG treatment, 8 genes were selected according to the criteria of either immune relevancy or common in a high percentage of samples for confirmation of expression data (see 3.5) and to show their progression during the time course (Fig. 2).

For probe-sets differentially regulated in the control-group, 7 genes were selected according to the criteria of either immune relevancy or common in a high percentage of samples for confirmation of expression data (see 3.5) and to show their progression during the time course (Fig. 3).

Table 4 Number of differentially expressed probe-sets on the HU-133A Genechip (Affymetrix) after IVIG treatment according to 2-fold induction/reduction criteria and a minimum of presence of at least 40% (4 out of 10) of patients;

Fold change in gene expression	Number of probe-sets affected by IVIG	
	Increases	Decreases
≥ 2–change in any patient	4403	8356
≥ 2–change in at least 40% of patients	26	104
≥ 2–change in at least 50% of patients	3	32
≥ 2–change in at least 60% of patients	0	11
≥ 2–change in at least 80% of patients	0	0
≥ 2–change in at least 100% of patients	0	0

Table 5 Number of differentially expressed probe-sets at the three different time-points of blood sampling after IVIG treatment; Criteria applied are a 2-fold change and a minimum of presence of at least 40% (4 out of 10 patients);

Differentially expressed genes			
≥ 2-fold changes in at least 40% of patients	comparison day 6 to day 0	comparison day 21 to day 0	comparison day 21 to day 6
increases	29	0	0
decreases	6	7	134
total	35	7	134
total all	176		

Table 6 Number of differentially expressed probe-sets on the HU-133A Genechip (Affymetrix) after IVMP treatment according to 2-fold induction/reduction criteria and a minimum of presence of at least 40% (2 out of 5) of control-patients;

Fold change in gene expression	Number of probe-sets affected by IVMP	
	Increases	Decreases
≥ 2–change in any patient	3178	6197
≥ 2–change in at least 40% of patients	204	494
≥ 2–change in at least 60% of patients	20	52
≥ 2–change in at least 80% of patients	7	7
≥ 2–change in at least 100% of patients	0	0

Table 7 Number of differentially expressed probe-sets at the three different time-points of blood sampling after IVMP treatment; Criteria applied are a 2-fold change and a minimum of presence of at least 40% (2 out of 5 control-patients);

Differentially expressed genes			
⩾ 2-fold changes in at least 40% of patients	comparison day 6 to day 0	comparison day 21 to day 0	comparison day 21 to day 6
increases	78	18	135
decreases	422	69	42
total	520	87	177
total all	784		

Figure. 2

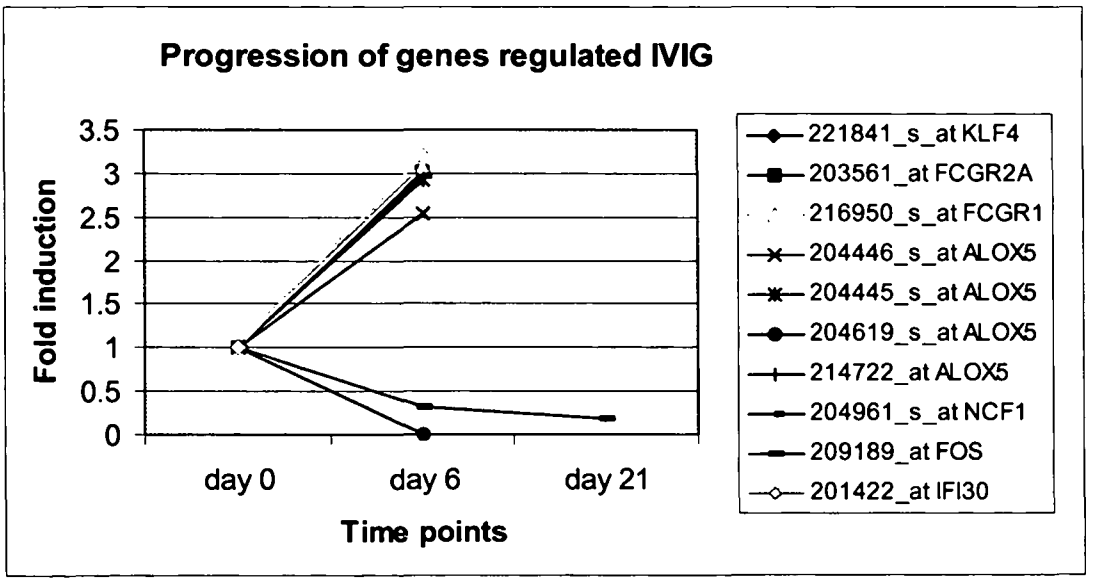


Figure 2a:
Progression of genes used for Real Time PCR confirmation under the influence of IVIG;
Microarray data are displayed as fold-inductions; Day 0 was set as 1;
Data are given for the following time points: Day 6 compared to day 0 and day 21 compared to day 0 after beginning of IVIG treatment;

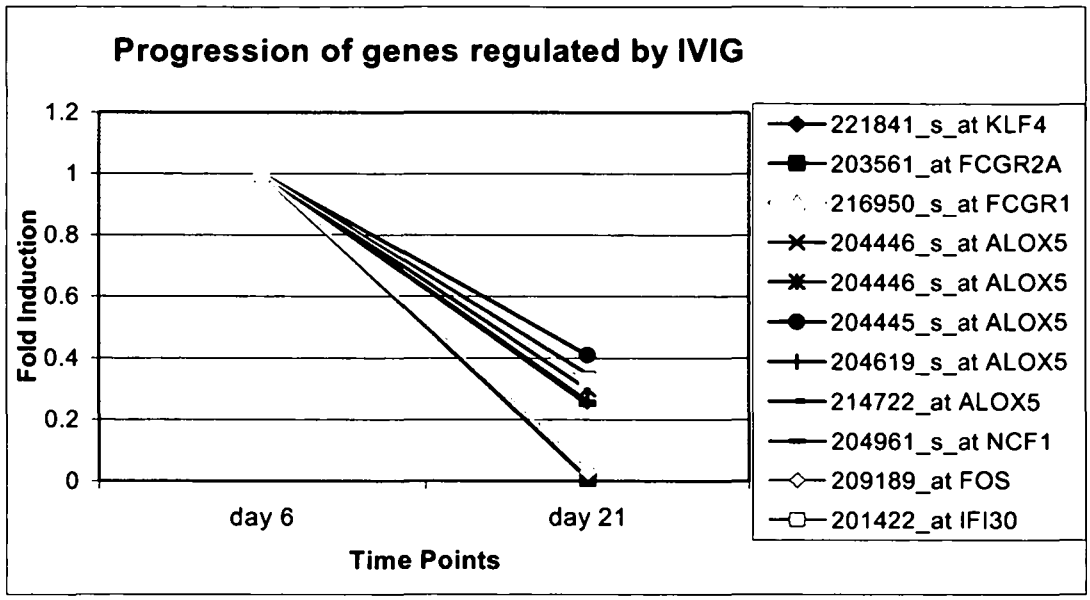


Figure 2b:
Progression of genes used for Real Time PCR confirmation under the influence of IVIG;
Microarray data are displayed as fold-inductions; Day 0 was set as 1;
Data are given for the following time points: Day 21 compared to day 6 after beginning of IVIG treatment;

Figure 3

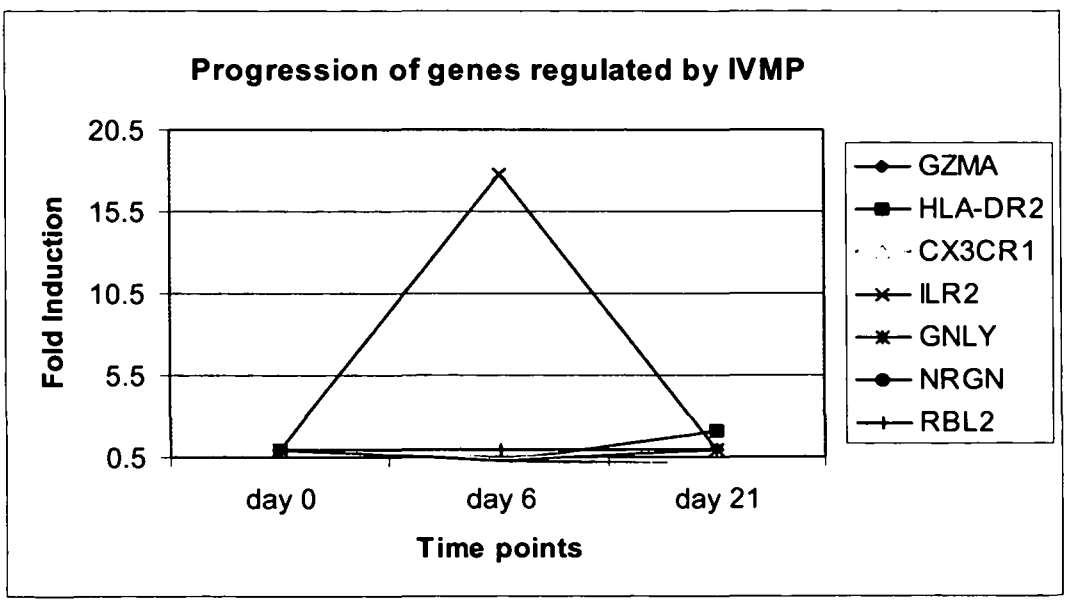


Figure 3a:
Progression of genes used for Real Time PCR confirmation under the influence of IVMP;Microarray data are displayed as fold-inductions; Day 0 was set as 1;
Data are given for the following time points: Day 6 compared to day 0 and day 21 compared to day 0 after beginning of IVMP treatment;

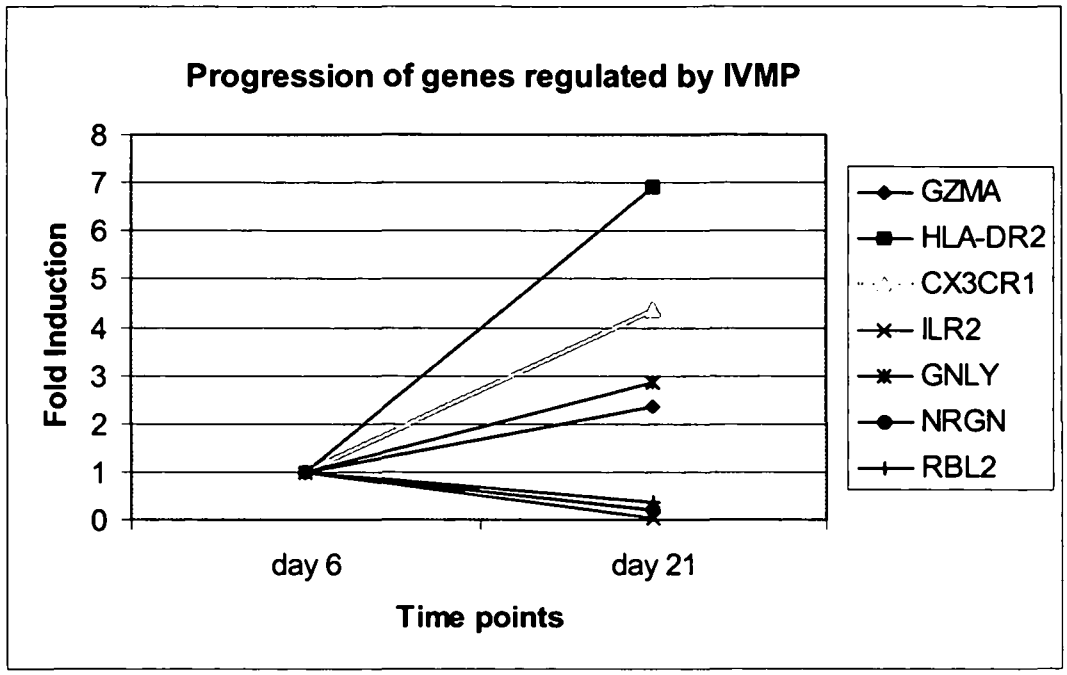


Figure 3b:
Progression of genes used for Real Time PCR confirmation under the influence of IVIG; Microarray data are displayed as fold-inductions; Day 0 was set as 1;
Data are given for the following time points: Day 21 compared to day 6 after beginning of IVIG treatment;

3.4.3 Statistical evaluation of gene expression data

Statistical analysis of the same data set consisting of microarray results of T cells of 10 patients suffering from MS in acute relapse gave a completely different number of significant differentially regulated genes: 360 probe-sets showed a change in expression upon IVIG treatment using a parametric t-test and 176 probe-sets showed a change using Data Mining Tool for analysis. In the control group treated with IVMP the parametric t-test revealed 583 probe-sets differentially regulated and the approach using Data Mining Tool showed 784 probe-sets of significantly differential regulated genes (Tab. 8 und Tab. 9).
The complete lists of all statistically significant genes differentially regulated upon IVIG and IVMP treatment are given in the attachment section (see Tab. A3 for IVIG and Tab. A4 for IVMP).
These list include immune or inflammatory related genes like PTGER4 (Prostaglandin E receptor 4), encoding a protein which is a member of the G-protein

coupled receptor family and being one of four receptors identified for prostaglandin E2 (PGE2), a receptor which can activate T-cell signalling or the IL11 gene encoding a protein which is a member of the gp130 family of cytokines and it is shown to stimulate the T-cell-dependent development of immunoglobulin-producing B cells and it is also found to support the proliferation of haematopoietic stem cells and megakaryocyte progenitor cells.

Probe-sets not involved in immune or inflammatory response function mainly in signalling, transcription, regulation of proliferation or cell cycle.

The two approaches for the analysis of differentially regulated genes only had 2 genes in common for patients treated with IVIG: STAT1 and CDKN1 (Tab. 10). For patients treated with IVMP the two approaches shared 5 different genes in common: IRF8, CX3CR1, GZMB, ILT7 and PTGDS (Tab. 11).

Table 8: Number of differentially regulated probe.sets after IVIG treatment obtained by the statistical approach; Data have a minimum of a 2-fold change in 100% of all patients (10 out of 10)

Differentially expressed			
≥ 2-fold changes in 100% of patients	compariso day 6 to day	compariso day 21 to	compariso day 21 to day
increase	52	71	54
decrease	39	76	68
total	91	147	122
total	360		

Table 9: Number of differentially regulated probe.sets after IVMP treatment obtained by the statistical approach; Data have a minimum of a 2-fold change in 100% of all patients (5 out of 5)

Differentially expressed			
⩾ 2-fold changes in 100% of patients	compariso day 6 to day	compariso day 21 to	compariso day 21 to day
increase	83	78	117
decrease	135	85	85
total	218	163	202
total	583		

Table 10: Comparison of the two methods for analyzing differentially expressed genes upon IVIG treatment;
Method 1: Analysis of data using Data Mining Tool software;
Criteria: minimum of 2-fold change in at least 40% of patients;
Method 2: Analysis of data using parametric t-test;
Criteria: minimum of 2-fold change in 100% of patients;
Intersection: genes in common by these two approaches;

Intersection of differentially expressed genes upon IVIG treatment by DMT-analysis and parametric t-test:

	Probe-Set ID	Biological Process
STAT1	209969_s_at	regulation of cell cycle, regulation of transcription, DNA-dependent transcription from RNA polymerase II promotor caspase activation intracellularsignaling cascade STAT protein nuclear translocation response to pest, pathogen or parasite
CDKN1	213348_at	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle cell cycle cell cycle arrest negative regulation of cell proliferation, negative regulation of cell cycle

Table 11: Comparison of the two methods for analyzing differentially expressed genes upon IVMP treatment;
Method 1: Analysis of data using Data Mining Tool software;
Criteria: minimum of 2-fold change in at least 60% of patients;
Method 2: Analysis of data using parametric t-test;
Criteria: minimum of 2-fold change in 100% of patients;
Intersection: genes in common by these two approaches;

Intersection of differentially expressed genes upon IVMP treatment by DMT-analysis and parametric t-test:

	Probe-Set ID	Biological Process
IRF8	204057_at	negative regulation of transcription from RNA polymerase II promotor regulation of transcription DNA-dependent immune response
CX3CR1	205898_at	chemotaxis cellular defense response cell adhesion signal transduction G-protein coupled receptor protein singalling pathway
GZMB	210164_at	proteolysis and peptidolysis apoptosis cleavage of lamin cytolysis
ILT7	210313_at	immune response
PTGDS	211748_x_at 212187_x_at	prostaglandin biosynthesis fatty acid biosynthesis transport regulation of circadian sleep/wake cycle

3.4.4 Comparison between patients treated with IVIG and the control group

To analyse if the differentially expressed genes obtained by microarray analysis are due to IVIG treatment or the pathologic MS background of the patients, a group of five patients was included as a control. These patients also suffered from MS in acute relapse, but were not treated with IVIG but intravenous methylprednisolone, a glucocorticoid used as an anti-inflammatory drug. By comparison of both expression-data sets, an exclusion of genes differentially regulated upon IVMP treatment only was possible. By performing an intersection of both gene lists, common genes differentially regulated upon immunomodulatory/immunosuppressive treatment in general were filtered out. The intersection between the genes obtained from IVIG treated patients and the genes obtained from IVMP treated patients revealed a list of 2 probe-sets in common (Tab. 12): MARCKS, coding for a protein involved in cell motility and KLF4 encoding a protein involved in the negative regulation of cell proliferation. KLF4 was increased at day 6 compared to day 0 in 50% of all patients during IVIG treatment and decreased again at day 21 compared to day 6 in 60% of all patients during IVIG and IVMP treatment. It plays a role in cell cycle regulation and epithelial differentiation. It is also an essential mediator of p53 in controlling G1/S progression of the cell cycle following DNA damage, thus acting as a tumor suppressor gene necessary for preventing the entry into mitosis following DNA damage. This is consistent with a clonal expansion of specific cell clones (T cells and B cells) which is a result of immune stimulation. Up-regulation of KLF4 amplifies the negative regulation of proliferation leading to immune suppression.

Table 12: Intersection (common genes) between IVIG treatment and IVMP treatment according to Data Mining
Criteria for IVIG treatment: a minimum of a 2-fold change in at least 60% of patients (6 out of 10);
Criteria for IVMP treatment: a minimum of a 2-fold change in at least 60% of patients (3 out of 5);

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location
201669_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	6q22.2
221841_s_at	KLF4	Kruppel-like factor 4 (gut)	9q31

The intersection between the two lists - genes differentially regulated upon IVIG and IVMP treatment - of the statistical approach using the t-test showed 18 genes in common (Table 13), most of them involved in intracellular signalling, replication and cell adhesion.

Table 13: Intersection (common genes) between IVIG treatment and IVMP treatment according to the parametric t-test
Criteria for IVIG treatment: a minimum of 2-fold change in 100% of patients;
Criteria for IVMP treatment: a minimum of 2-fold change in 100% of patients;

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location
202837_at	FLN29	FLN29 gene product	12q
203862_s_at	ACTN2	actinin, alpha 2	1q42-q43
204677_at	CDH5	cadherin 5, type 2, VE-cadherin (vascular epithelium)	16q22.1
205085_at	ORC1L	origin recognition complex, subunit 1-like (yeast)	1p32
205151_s_at	KIAA0644	KIAA0644 gene product	7p15.1
206175_x_at	ZNF222	zinc finger protein 222	19q13.2
208546_x_at	HIST1H2BH	histone 1, H2bh	6p21.3
209819_at	HABP4	hyaluronan binding protein 4	9q22.3-q31
209969_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	2q32.2
210415_s_at	ODF2	outer dense fiber of sperm tails 2	9q34.11
213991_s_at	HS3ST1	Heparan sulfate (glucosamine) 3-O-sulfotransferase 1	4p16
215409_at	LOC254531	PLSC domain containing protein	15q14
215767_at	C2orf10	chromosome 2 open reading frame 10	2q32.1
216814_at	---	---	---
217082_at	---	Unknown protein	---
219534_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5
220425_x_at	ROPN1B	ropporin, rhophilin associated protein 1B	3q21.2
222258_s_at	SH3BP4	SH3-domain binding protein 4	2q37.1-q37.2

Subtraction of genes differentially regulated upon IVMP treatment from genes differentially expressed upon IVIG treatment revealed 11 IVIG-specific probe-sets or 9 different genes (Tab. 14). Examples are FCGR2A involved in immune response or ALOX5 involved in inflammatory response.

The Fc fragment of IgG, the low affinity receptor IIa (FCGR2A, CD32), was up-regulated at day 6 compared to day 0 and became down-regulated again at day 21 compared to day 6. Receptors for the Fc portion of IgG play an essential role in the protection of the organism against foreign antigens by removing antigen-antibody complexes from the circulation. Receptors are present on T and B lymphocytes, monocytes, macrophages, neutrophils and natural killer (NK) cells. The receptor may also act in an inhibitory way terminating activation signals via the ITIM-motives in the cytoplasmatic tail. An up-regulation of this Fc receptor after IVIG treatment, which consist of > 90% of IgG seems to be a normal consequence of the input of human antibodies into the circulation. As the IVIG-antibodies become cleared out of the body, the receptor becomes down-regulated again. By binding of IVIG antibodies to the receptor, inhibitory signal may act on the acute inflammatory response taking place in the CNS during the relapse, thus down-regulating the acute immune response.

FOS is a member of the FOS gene family and can dimerize with members of the JUN family, thereby forming the transcription factor complex AP-1. FOS is a regulator of cell proliferation, differentiation, transformation and has also been associated with apoptotic cell death. FOS/JUN regulate themselves. Influences from outside increase their synthesis. For example c-FOS is induced at the beginning of cell proliferation. The activity of FOS/JUN can also be regulated by the transfer of phosphate-groups on amino-acids in either the transactivation-domain or the DNA-binding-domain. The transcription factor AP-1 is one of the three factors activated in T cells by antigen recognition via TCR-signaling. Activation of AP-1 leads to transcription of IL-2. IL-2 is responsible for the proliferation of antigen-specific T cells, promotes proliferation and differentiation of other immune cells and potentiates apoptotic death of antigen- activated T cells. FOS is down-regulated in 60% of all patients at day 21 compared to day 6.

Down-regulation of FOS leads to down-regulation of AP-1 followed by decreased induction of IL-2 resulting in decreased proliferation of immune cells. This finding is in line with the immunosuppresent effect of IVIG on immune cells.

Here IVIG seems to induce a long-term effect on the down-regulation of immune cells thus maybe preventing the further entry of autoreactive T cells into the CNS, which causes the relapses of the patients.

Table 14: Subtraction of IVMP-specific genes from IVIG-specific genes according to the DMT-approach; Criteria: minimum of a 2-fold change in at least 60% of patients: 6 out of 10 for the IVIG group; 3 out of 5 for the IVMP group;

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location
201422_at	---	---	---
201798_s_at	FER1L3	fer-1-like 3, myoferlin (C. elegans)	10q24
203561_at	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	1q23
204445_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2
204446_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2
208018_s_at	HCK	hemopoietic cell kinase	20q11-q12
208890_s_at	PLXNB2	plexin B2	22q13.33
209189_at	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	14q24.3
210873_x_at	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	22q13.1-q13.2
214366_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2
214511_x_at	---	---	---

Using the statistical approach, there were 253 probe-sets specific for IVIG treatment after subtraction of the probe-sets differentially regulated upon IVMP therapy (Tab. A6, see attachment) like for example the chemokine ligands CXCL3 and CXCL5.

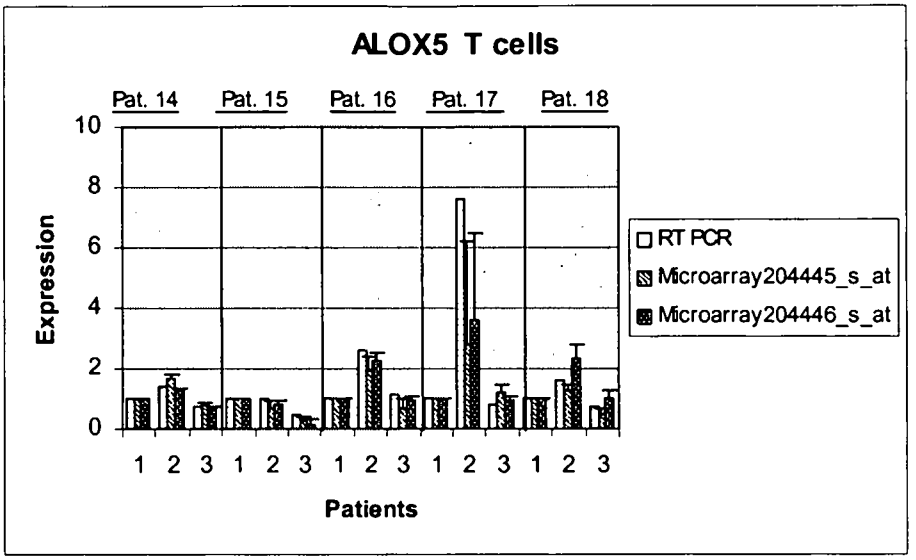
3.4.5 Confirmation of microarray data by Real-time PCR

Microarray analysis was confirmed by quantitative Real-time PCR,
using primers for 8 genes as an example according to the criteria of either immune relevancy or common in a high percentage of samples (at least in a minimum of 50% of all patients): Fc fragment of IgG, low affinity IIa-receptor (FCGR2A), Fc fragment of IgG, high affinity Ia, receptor (FCGR1A), arachidonate 5-lipoxygenase (ALOX5), v-fos murine osteosarcoma viral oncogene homolog (FOS), neutrophil cytosolic factor 1 (NCF1), interferon, gamma-inducible protein 30 (IFI30) and Kruppel-like factor 4 (KLF4) for the analysis performed using the 2-fold filtering criteria (Fig. 4a-h).

Microarray results are given in fold-induction in comparison to Real Time PCR results that are given in relative expression normalized to an endogenous control according to the ^^CT-method. Therefore an exact comparison of both data sets is not possible, but the trend of an up- or down-regulation should be the same in both methods (13). Indeed, Real Time PCR results could confirm the microrarray data. Discrepancies were only seen for ALOX5/patient 17.2, FOS/patient 16.2 and 17.2, NCF1/patient 18.2, FCGR2A/ patient 18.2 and FCYR1/patient 16.3 and 17.2 . FCYR1 is annotated by two probe sets: 216950_s_at and 214511_x_at. The discrepancy can also be due to the x-annotation — which allows cross-hybridization with other genes – or the alternative splice site of the s-annotation. Most differences were seen at day 6 compared to day 0 after IVIG treatment. As analysis was performed with patient material, one has to consider that patients are a very heterogeneous group, all having a different immunological state and reacting in a different way to IVIG.

Figures 4a-c:

a)



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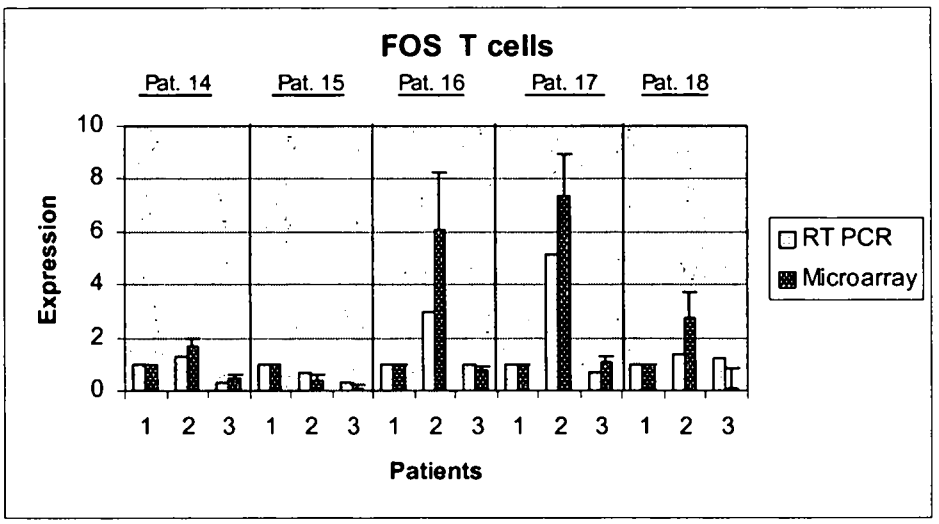

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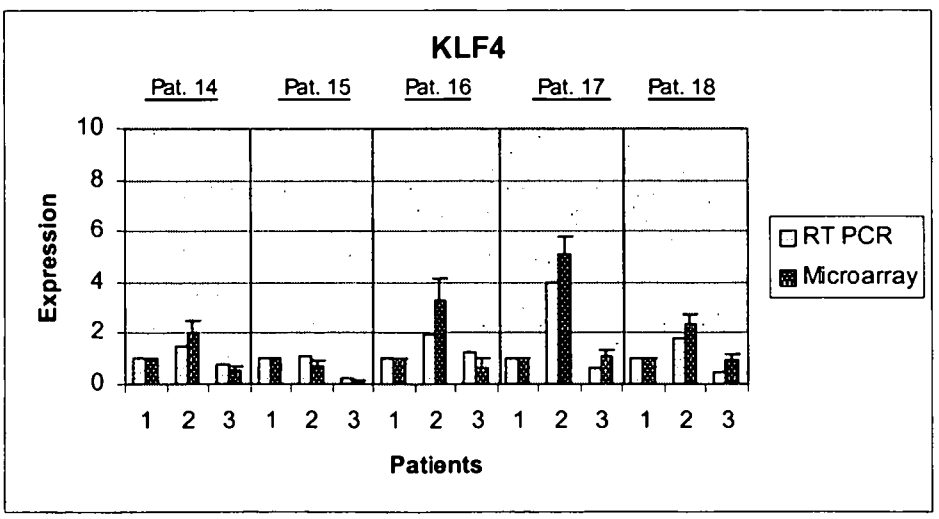
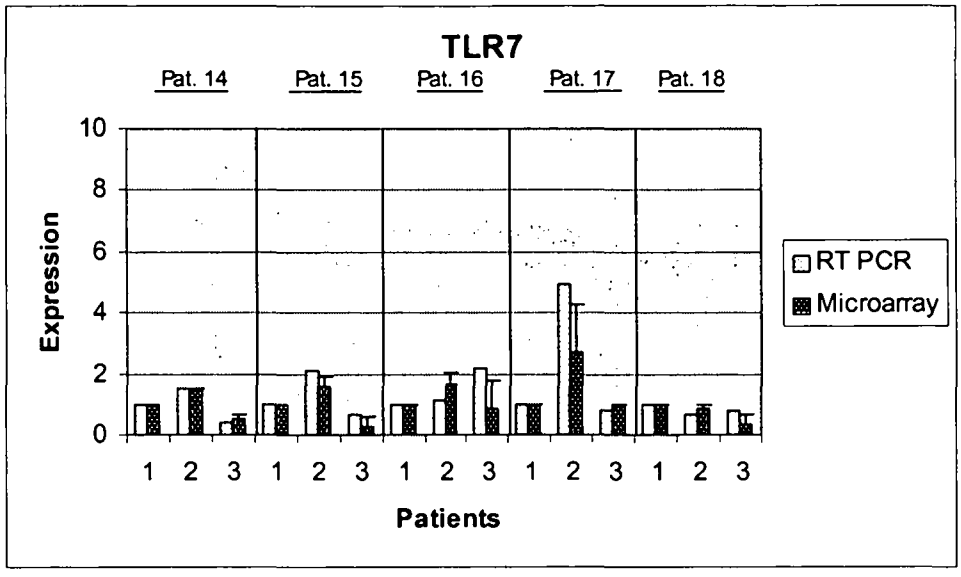


Figures 4d-f:

d)



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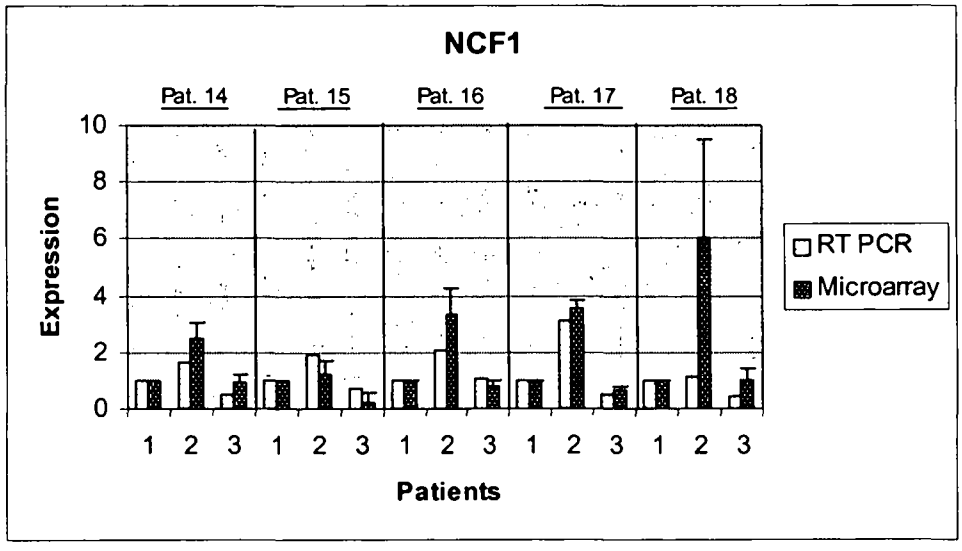

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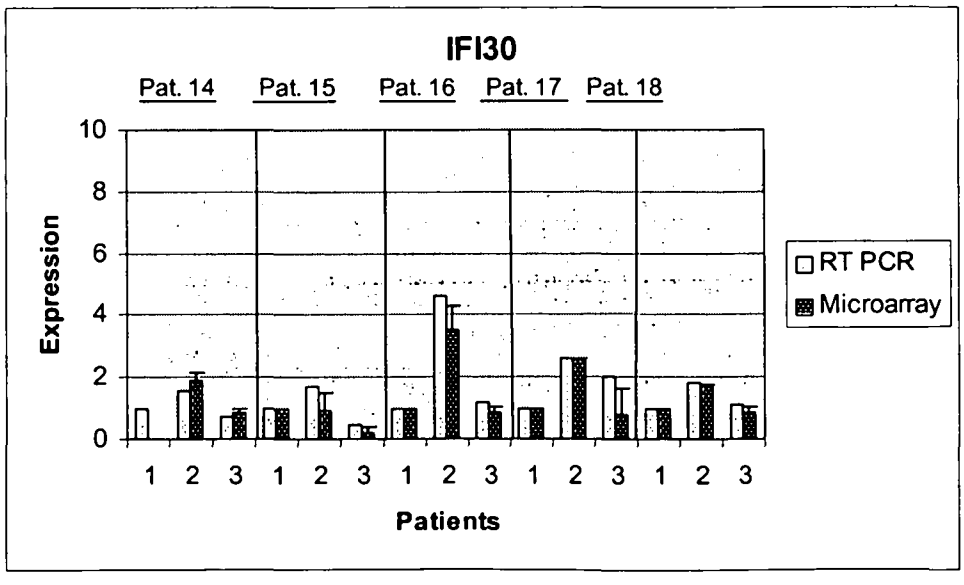
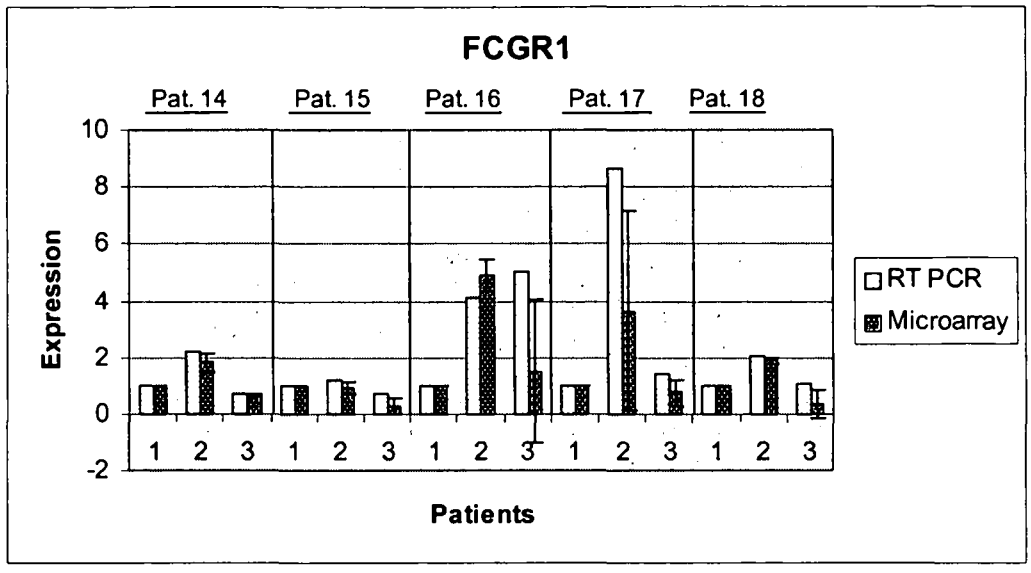
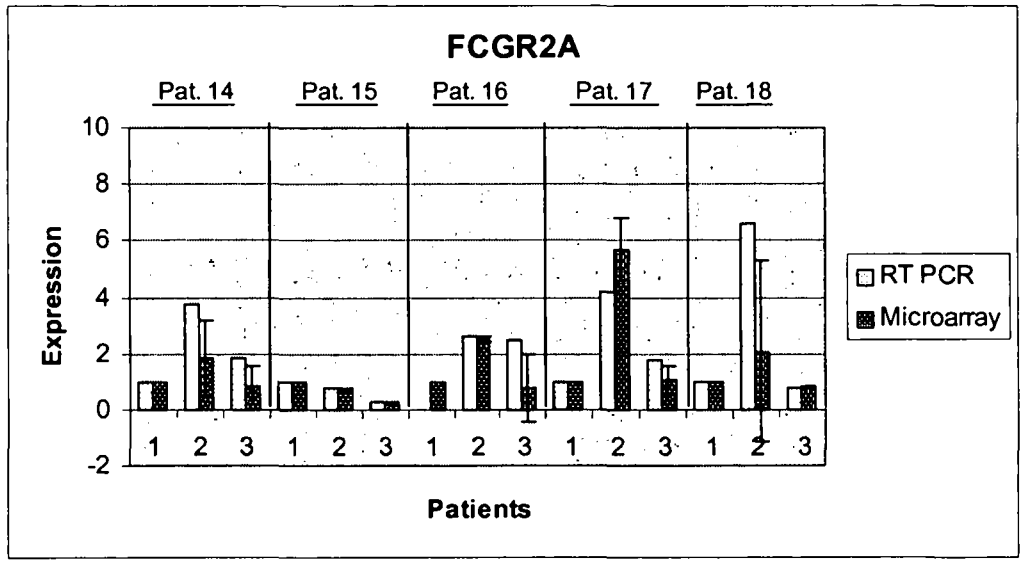


Figures 4g-h:

g)



h)



Figures 4a-h:
Verification of differential expression of genes affected by IVIG treatment of MS-patients in acute relapse by Real Time PCR. All primers used were pre-designed assays-on-demand. Presented data are given in fold-changes for microarray results and in relative expression normalized to the endogenous control (GPPH) for PCR results. Standard deviation is given for comparison of both data sets. Real Time experiments were taken in triplets and confirmed by a second round.

For confirmation of expression of the genes in the group treated with IVMP, 7 genes were confirmed by Real Time PCR as an example using primers for the following genes according to the criteria of either immune relevancy or common in a high percentage of samples: GZMA, HLA-DRA, CX3CR1, ILR2, GNLY, NRGN and RBL2. The trend towards an increase or a decrease in the differential regulation of microarray and Real Time PCR could be confirmed (Figures 5a-g).

Only with the gene RBL2 there were some noticeable differences between expression fold-changes. Patient 8.2 showed an increase in expression by 1.6-fold in Real Time PCR but by 2-fold on the micorarray. The most amazing increases were seen for the IL1R2 gene at day 6 compared to day 0 after IVMP treatment. It was increased by >10-fold in Real Time PCR (patient 7.2) and by >20-fold on the microarray as well as by >25-fold in Real Time PCR (patient 8.2) and >30-fold on the microarray.

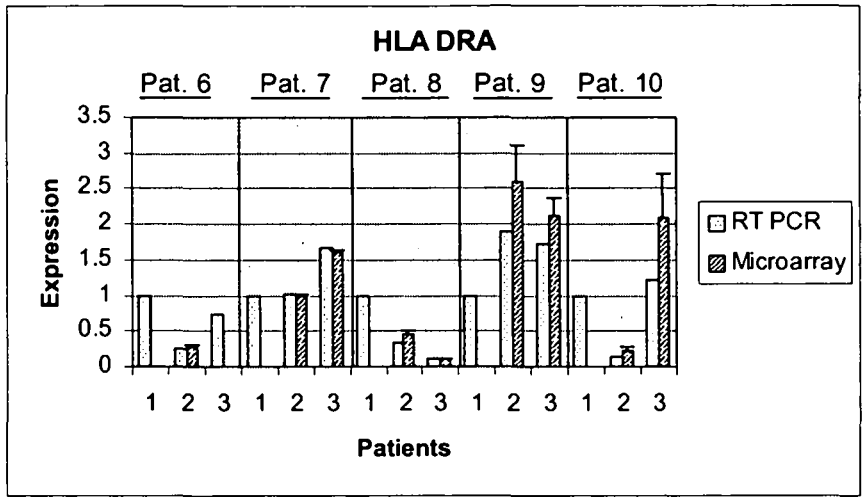
Another gene which was highly up-regulated in one patient (#9) was NRGN.

At day 6 compared to day 0 after IVMP treatment, it was up-regulated by ~ 10-fold and at day 21 compared to day 0 it was down-regulated again by 5-fold.

Unfortunately there was no micorarray signal for patient # 6 at day 21 after beginning of treatment (time point 6.3) for all genes selected for confirmation. This could be due to unsuccessful binding of target cRNA to the probes, or even bad quality of cRNA by itself. Therefore, it was impossible to compare results obtained by Real Time PCR at day 21 with the microarray results for patient 6.3.

Figures 5a-c:

a)



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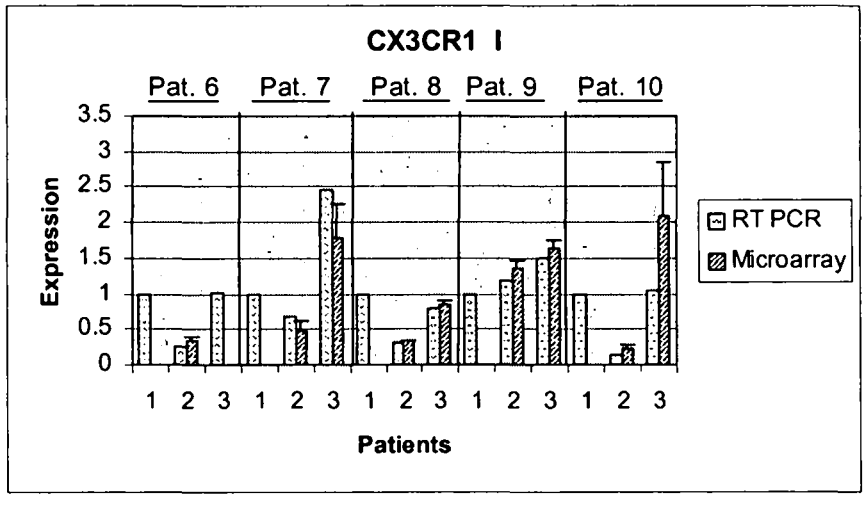
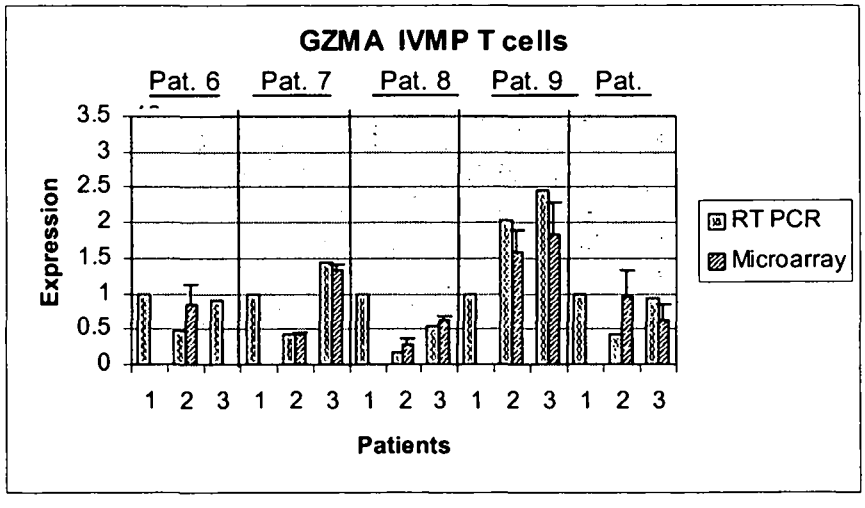
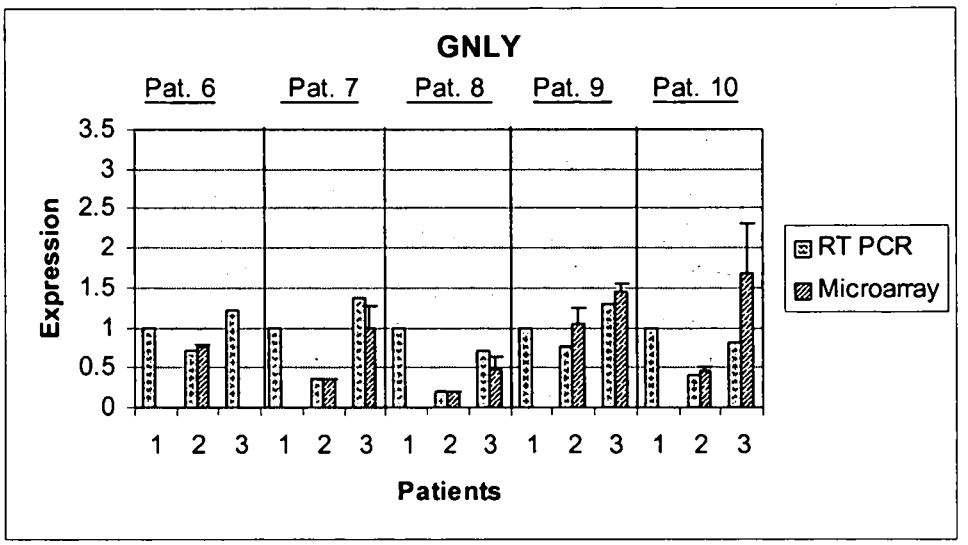


c)

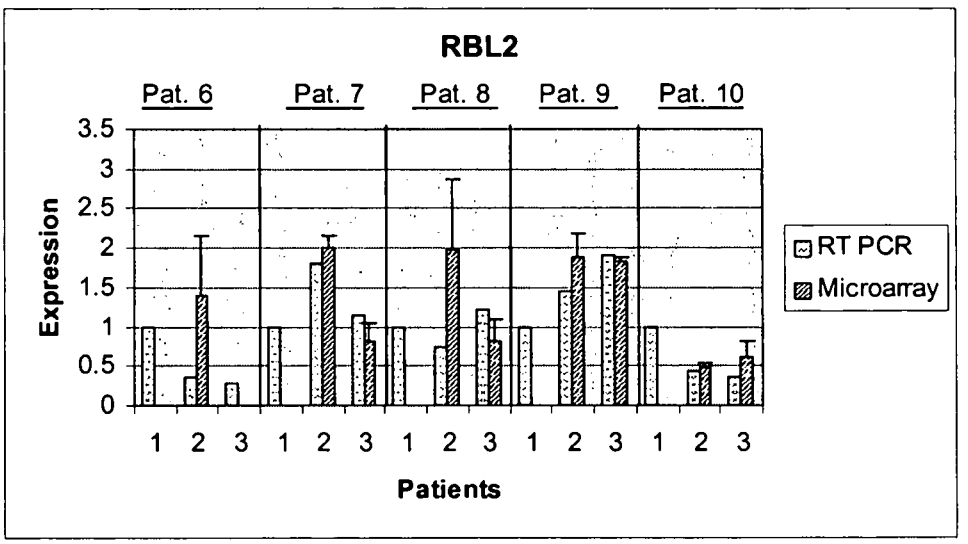


Figures 5d-e:

d)

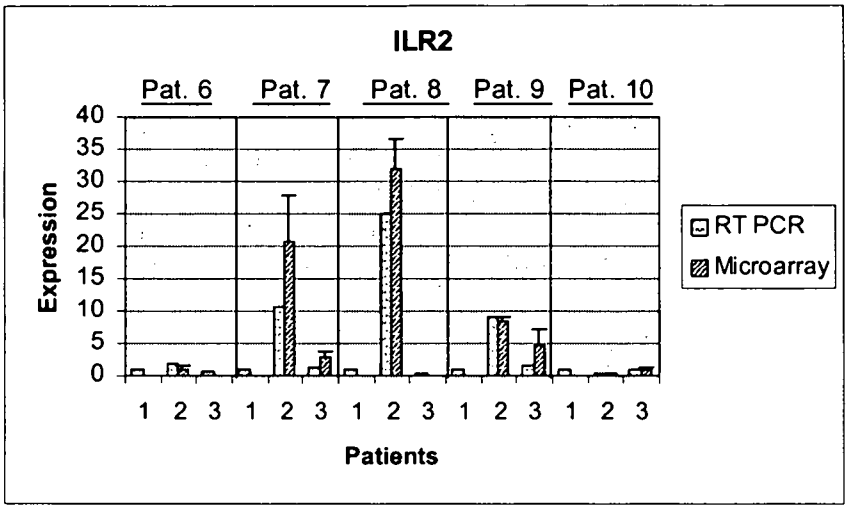


e)

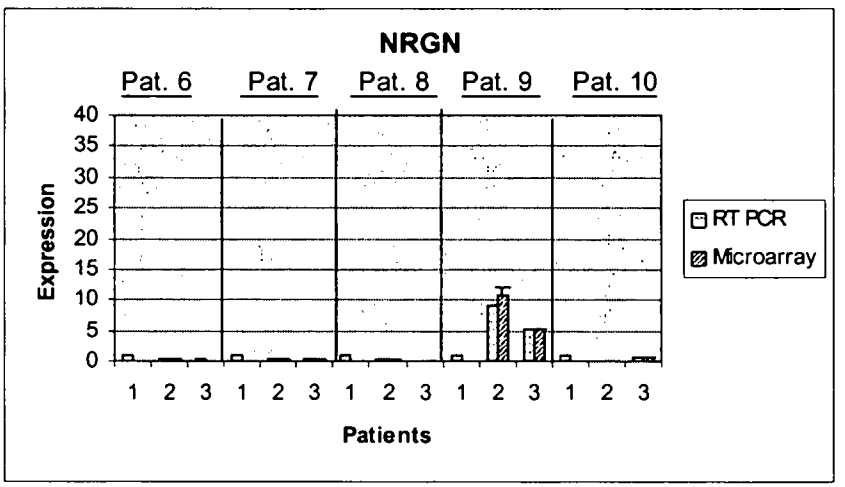


Figures 5f-g:

f)



g)



Figures 5a-g:
Verification of differential expression of genes affected by IVMP treatment of MS-patients in acute relapse by Real Time PCR. All primers used were pre-designed assays-on-demand. Presented data are given in fold-changes for microarray results and in relative expression normalized to the endogenous control (GPPH) for PCR results. Standard deviation is given for comparison of both data sets. Real Time experiments were taken in triplets and confirmed by a second round. Figures 3f and 3g use a different scale due to high increases;

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4.1 Discussion

Multiple sclerosis is the most common chronic inflammatory neurological disease. Despite major advances in aetiology of this disease, it is still not completely understood. Epidemiological studies and magnetic resonance imaging have provided important inside into the natural course and prognostic factors of MS, but the ability to predict different courses of the disease, and especially its response to treatment, is still very limited (1).

Peripheral blood mononuclear cells, in particular peripheral T cells, have been shown to be involved in the disease pathogenesis and induce active demyelination. Recently it was shown that a number of genes in peripheral blood mononuclear cells of patients were differentially expressed when compared to healthy subjects (2). These results support the involvement of peripheral cells in the pathogenesis of the disease.

The present study was designed to identify genes differentially expressed in T cells of patients with RRMS in acute exacerbation after treatment with IVIG. The clinical outcome of the study showed that IVIG given in a 5-day course at 0.4g/kg per day was effective and caused significant changes in EDSS score as well as changes in disease activity. Furthermore, the treatment was safe and well tolerated.

Using GCOS- and Data Mining Tool software for data analysis, I found 176 genes to be at least 2-fold up- or down-regulated in a minimum of 40% of all patients (4 out of 10). The majority of these genes was down-regulated (147) and the differential regulation occurred mostly at day 21 compared to day 6 after beginning of IVIG therapy. Most of the proteins encoded by these genes are involved in the regulation of immune- and inflammatory responses, signal transduction, transcription, apoptosis or cell proliferation. Prominent examples are genes coding for chemokines or chemokine ligands that are involved in the regulation of leukocyte migration, genes coding for proteins involved in synthesis and regulation of prostaglandins, genes coding for toll-like receptors, genes coding for Fc receptors or genes coding for cytokines.

All these biological activities are likely to be involved in the regulation of disease activity in patients with RRMS. To differentiate between genes regulated upon

IVIG treatment and genes differentially expressed due to the pathologic background of MS, we included a control group treated with an immunosupressive drug (IVMP) that represents the current standard of care. There were 784 genes differentially regulated in peripheral T cells upon IVMP treatment showing at least a 2-fold change in expression. The majority of these genes were down-regulated, most often at day 6 compared to day 0 after beginning of therapy. When I compared differentially expressed genes found in peripheral T cells under IVIG treatment with those found under IVMP treatment, I found a total of 133 genes to be regulated only in patients treated with IVIG. Therefore, I believe that I have identified a set of genes that might be associated with the biological activity of IVIG in patients with RRMS. Further evaluation of these genes in a larger group of patients will be necessary to confirm our data and to eventually define a set of differentially expressed genes that can be clearly associated with the clinical efficacy of IVIG in RRMS.

Statistical analysis using a parametric-t test gave a rather different result when compared to the analysis using GCOS- and Data Mining Tool software. A total of 360 genes were differentially expressed with a minimum of a 2-fold change in expression. Both methods had only 2 genes in common: STAT1 and CDKN1, both encoding proteins that are involved in intracellular signalling or cell cycle regulation. There might be two reasons for the observed differences. First, our approach using the Data Mining Tool Software was more restrictive, having the criteria of either 2-fold increase or decrease, a minimum of change in at least 40% of all patients, a requirement for present calls of 100% as well as a p-value cut off of 0.004 or 0.096.

The statistical approach had a minimum requirement for present calls in at least 50% of patients and used data of all 100% of patients. Second, it is a well-known problem in the literature, that comparing different methods for statistical analysis of microarray data can give rather conflicting results (3-5).The issue of high dimensionality in microarray data has been, and remains, a hot topic in statistical and computational analysis. Efficient gene filtering and differentiation approaches can reduce the dimension of data, help to remove redundant genes and noises, and highlight the most relevant genes that are major players in the development of certain diseases or the effect of drug treatment. A significant body of data on gene

expression patterns in autoimmune diseases such as MS has been generated by microarray analysis (1;3-18). Although the results are considered to be very promising, there are many factors that have detracted from the data. No common methodological directions are available. Collection techniques, processing methods, and statistical approaches are often very different (19).
Recently, Liang et al (3) investigated the efficiency of parametric, non-parametric and semi-parametric gene filtering methods through the application of time course microarray data from MS patients being treated with interferon-beta-1a. Their results show that the presented methods used for the analysis of data performed significantly differently from each other. Therefore, commonly approved standard-guidelines for the statistical evaluation of gene expression data would be helpful for better reproducibility and comparability in the evaluation of microarray data.

Another problem with our clinical study was the small number of patients included (10 patients in the group treated with IVIG and 5 patients in the group treated with IVMP). Clearly, our data have to be confirmed in a larger study.

In conclusion, treatment of patients suffering from RRMS in acute exacerbations with IVIG showed beneficial effects, as shown by the significant reduction of EDSS scores and by the significant reduction in active brain lesions illustrated by MRI. IVIG had a significant down-regulatory effect on many genes involved in immune or inflammatory responses. Selection of the most important genes affected by IVIG could help in establishing a clinically relevant micorarray for testing the immunomodulatory effects of IVIG in RRMS.

Gene expression data in MS

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Tab A1:

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location	GO Biological Process
201422_at	IFI30	interferon, gamma-inducible protein 30	19p13.1	immune response
203561_at	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	1q23	immune response
209189_at	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	14q24.3	DNA methylation, regulation of transcription from RNA polymerase II promoter, inflammatory response
214511_x_at	FCGR1	Fc fragment of IgG, low affinity Ia, receptor (CD64)	1q21.2-q21.3	phagocytosis, engulfment, immune response, signal transduction
214366_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
221841_s_at	KLF4 ↑	Kruppel-like factor 4 (gut)	9q31	transcription, mesoderm cell fate determination, negative regulation of cell proliferation
201669_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	6q22.2	cell motility
201798_s_at	FER1L3	fer-1-like 3, myoferlin (C. elegans)	10q24	muscle contraction, circulation
208018_s_at	HCK	hemopoietic cell kinase	20q11-q12	protein amino acid phosphorylation, intracellular signaling cascade, mesoderm development
208890_s_at	PLXNB2	plexin B2	22q13.33	development
210873_x_at	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like3A	22q13.1-q13.2	---

201743_at	CD14	CD14 antigen	5q22-q32/5q31.1	phagocytosis; apoptosis; inflammatory response; cell surface receptor linked signal transduction
204445_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
204533_at	CXCL10	chemokine (C-X-C motif) ligand 10	4q21	cell motility; chemotaxis; inflammatory response; cell surface receptor linked signal transduction
				cell-cell signaling; muscle development sensory perception; circulation
				positive regulation of cell proliferation
216950_s_at	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	1q21.2-q21.3	phagocytosis, engulfment; immune response
219519_s_at	SN	sialoadhesin	20p13	inflammatory response, cell-matrix adhesion, cell-cell adhesion
220146_at	TLR7	toll-like receptor 7	Xp22.3	inflammatory response, immune response
204858_s_at	PDGFL	endothelial cell growth factor 1 (platelet-derived)	22q13.31	mitochondrial genome maintenance, angiogenesis; pyrimidine base metabolism; chemotaxis
				pyrimidine nucleotide metabolism; DNA replication; cell surface receptor linked signal transduction
				cell-cell signaling; sensory perception; metabolism; cell differentiation
204961_s_at	NCF1	neutrophil cytosolic factor 1	7q11.23	electron transport; superoxide metabolism; cellular defense response, intracellular signaling cascade
		(47kDa, chronic granulomatous disease, autosomal 1)		
217763_s_at	RAB31	member RAS oncogene family	18p11.3	small GTPase mediated signal transduction
220088_at	C5R1	complement component 5 receptor 1 (C5a ligand)	19q13.3-q13.4	activation of MAPK cheMotaxis; G-protein coupled receptor protein signaling pathway
				phospholipase C activation; positive regulation of cytosolic calcium ion concentration
				sensory perception of chemical stimulus
218559_s_at	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B	20q11.2-q13.1	transcription; regulation of transcription, DNA-dependent; sensory organ development
204439_at	IFI44 ↑	interferon-induced protein 44-like	1p31.1	---
214084_x_at	— ↑	---	---	---
201360_at	CST3	cystatin C	20p11.21	
201670_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	6q22.2	cell motility
202510_s_at	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	14q32	angiogenesis; cell differentiation
211429_s_at	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A	14q32.1	acute-phase response
		(alpha-1 antiproteinase, antitrypsin), member 1		
202833_s_at	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A	14q32.1	acute-phase response
		(alpha-1 antiproteinase, antitrypsin), member 1		
205936_s_at	HK3	hexokinase 3 (white cell)	5q35.2	glycolysis
206488_s_at	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	7q11.2	lipid metabolism; fatty acid metabolism; transport; cell adhesion; blood coagulation
213472_a	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)	5q35.3	RNA processing
219788_at	PILRA	paired immunoglobin-like type 2 receptor alpha	7q22.1	transmembrane receptor protein tyrosine kinase, activation (dimerization)

200986_at	SERPING1 ↑	serine (or cysteine) proteinase inhibitor	11q12-q13.1	immune response, complement activation, classical pathway, blood coagulation, circulation
		clade G (C1 inhibitor), member 1, (angioedema, hereditary)		
202269_x_at	GBP1 ↑	guanylate binding protein 1, interferon-inducible, 67kDa	1p22.2	immune response
203066_at	GALNAC4S ↑	B cell RAG associated protein	10q26	regulation of DNA recombination, regulation of B-cell differentiation
203535_at	S100A9 ↑	S100 calcium binding protein A9 (calgranulin B)	1q21	inflammatory response, cell-cell signaling
204415_at	G1P3 ↑	interferon, alpha-inducible protein (clone IFI-6-16)	1p35	immune response, response to pest, pathogen or parasite
204446_s_at	ALOX5 ↑	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
204747_at	IFIT3 ↑	interferon-induced protein with tetratricopeptide repeats 3	10q24	immune response
205483_s_at	G1P2 ↑	interferon, alpha-inducible protein (clone IFI-15K)	1p36.33	protein modification, immune response, cell-cell signaling
214146_s_at	PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	4q12-q13	regulation of cell cycle, chemotaxis, immune response, sensory perception
				cell proliferation, glucose transport, defense response to bacteria
203066_at	GALNAC4S-6ST	B cell RAG associated protein	10q26	regulation of DNA recombination, hexose biosynthesis, regulation of B-cell differentiation
203153_at	IFIT1			(sensu Vertebrata)
203535_at	S100A9	interferon-induced protein with tetratricopeptide repeats 1	10q25-q26	immune response
203922_s_at	CYBB	S100 calcium binding protein A9 (calgranulin B)	1q21	inflammatory response, cell-cell signaling
		cytochrome b-245, beta polypeptide (chronic granulomatous disease)	Xp21.1	electron transport, ion transport, inflammatory response, antimicrobial humoral response
203923_s_at	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	Xp21.1	(sensu Vertebrata)
				electron transport, ion transport, inflammatory response, antimicrobial humoral response
				(sensu Vertebrata)
204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	1q23	immune response, cell surface receptor linked signal transduction
207104_x_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B		
		(with TM and ITIM domains), member 1	19q13.4	immune response, response to virus
204924_at	TLR2	toll-like receptor 2	4q32	induction of apoptosis, inflammatory response, signal transduction
205715_at	BST1	bone marrow stromal cell antigen 1		regulation of transcription, DNA-dependent
205789_at	CD1D	CD1D antigen, d polypeptide	4p15	humoral immune response, development
			1q22-q23	detection of bacteria, T-cell selection, positive regulation of innate immune response,
205863_at	S100A12	S100 calcium binding protein A12 (calgranulin C)	1q21	antigen presentation, endogenous peptide antigen, antigen presentation, endogenous lipid antigen
				xenobiotic metabolism, inflammatory response, defense response to bacteria,
206380_s_at	PFC	properdin P factor, complement		defense response to fungi
206710_s_at	EPB41L3	erythrocyte membrane protein band 4.1-like 3	Xp11.3-p11.23	immune response, complement activation, alternative pathway, defense response to bacteria
209906_at	C3AR1	complement component 3a receptor 1	12p13.31	cell motility, chemotaxis, smooth muscle contraction, inflammatory response,
				cellular defense response, signal transduction, G-protein coupled receptor protein signaling pathway,
				neuropeptide signaling pathway, positive regulation of cytosolic calcium ion concentration,
				sensory perception, circulation
207857_at	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A	19q13.4	immune response, signal transduction
		(with TM domain), member 2		
208594_x_at	LILRB6	leukocyte immunoglobulin-like receptor, subfamily B	19q13.4	---
		(with TM and ITIM domains), member 6		
209500_x_at	TNFSF13	tumor necrosis factor (ligand) superfamily, member 13	17p13.1	immune response, signal transduction, positive regulation of cell proliferation
209555_s_at	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	7q11.2	lipid metabolism, fatty acid metabolism, transport, cell adhesion, blood coagulation
210629_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis,
				negative regulation of lymphocyte proliferation
210660_at	LILRA1	leukocyte immunoglobulin-like receptor, subfamily A	19q13.4	immune response, cell surface receptor linked signal transduction
		(with TM domain), member 1		
210895_s_at	CD86	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	3q21	immune response, cell-cell signaling, positive regulation of cell proliferation, T-cell activation, positive
				regulation of tumor necrosis factor-beta biosynthesis, positive regulation of interleukin-2 biosynthesis,
				positive regulation of interleukin-4 biosynthesis, positive regulation of T-helper 2 cell differentiation,
				positive regulation of transcription
210982_s_at	HLA-DRA	major histocompatibility complex, class II, DR alpha	6p21.3	immune response, immune response, antigen presentation, exogenous antigen,
				antigen processing, exogenous antigen via MHC class II
211336_x_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B	19q13.4	immune response, response to virus
211582_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis,
				negative regulation of lymphocyte proliferation
213716_s_at	SECTM1	secreted and transmembrane 1	17q25	immune response, mesoderm development, positive regulation of I-kappaB kinase/NF-kappaB cascade
214038_at	CCL8	chemokine (C-C motif) ligand 8	17q11.2	calcium ion transport, exocytosis, chemotaxis, inflammatory response, signal transduction,
				cell cycle arrest,
215633_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis,
				negative regulation of lymphocyte proliferation
221698_s_at	CLEC7A	C-type lectin domain family 7, member A	12p13.2-p12.3	phagocytosis, recognition, cell recognition, carbohydrate mediated signaling,
				antibacterial humoral response (sensu Vertebrata), antifungal humoral response (sensu Vertebrata),
				T-cell activation, defense response to pathogenic protozoa, reduction of virulence
221060_s_at	TLR4	toll-like receptor 4	9q32-q33	inflammatory response, signal transduction, activation of NF-kappaB-inducing kinase, detection of
				pathogenic bacteria, detection of fungi, T-helper 1 type immune response, macrophage activation,
				positive regulation of interleukin-12 biosynthesis, positive regulation of interleukin-1 biosynthesis,
				positive regulation of interleukin-13 biosynthesis, positive regulation of interleukin-6 biosynthesis
				mast cell activation, negative regulation of osteoclast differentiation
200829_at	WARS ↑	tryptophanyl-tRNA synthetase	14q32.31	protein biosynthesis, tryptophanyl-tRNA aminoacylation, negative regulation of cell proliferation
208891_at	DUSP6 ↑	dual specificity phosphatase 6	12q22-q23	regulation of cell cycle, inactivation of MAPK, protein amino acid dephosphorylation
201506_at	TGFBI	transforming growth factor, beta-induced, 68kDa	5q31	cell adhesion, negative regulation of cell adhesion, sensory perception, visual perception,
				cell proliferation
210148_at	HIPK3	homeodomain interacting protein kinase 3	11p13	transcription, regulation of transcription, DNA-dependent, protein amino acid phosphorylation,
				apoptosis
213524_s_at	G0S2	putative lymphocyte G0/G1 switch gene	1q32.2-q41	regulation of cell cycle
200678_x_at	GRN	granulin	17q21.32	negative regulation of cell proliferation
201041_s_at	DUSP1	dual specificity phosphatase 1	5q34	protein amino acid dephosphorylation, response to oxidative stress, cell cycle

207540_s_at	SYK	spleen tyrosine kinase	9q22	
				protein complex assembly, protein amino acid phosphorylation, leukocyte cell adhesion,
				integrin-mediated, signaling pathway, intracellular signaling cascade, cell proliferation,
				cell proliferation, organogenesis, neutrophil chemotaxis
213182_x_at	CDKN1C	erythrocyte membrane protein band 4.1-like 3	18p11.32	cortical actin cytoskeleton organization and biogenesis
		cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle,
				cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
216894_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle,
				cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
219534_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle,
				cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
208018_s_at	HCK	hemopoietic cell kinase	20q11-q12	protein amino acid phosphorylation, intracellular signaling cascade, mesoderm development
202626_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	8q13	protein amino acid phosphorylation, intracellular signaling cascade
204858_s_at	ECGF1	endothelial cell growth factor 1 (platelet-derived)	22q13]22q13.33	mitochondrial genome maintenance, angiogenesis, pyrimidine base metabolism
				pyrimidine nucleotide metabolism, DNA replication, chemotaxis, cell surface receptor linked
				signal transduction, cell-cell signaling, sensory perception, metabolism, cell differentiation
220088_at	C5R1	complement component 5 receptor 1 (C5a ligand)	19q13.3-q13.4	activation of MAPK, chemotaxis, cellular defense response, signal transduction,
				G-protein coupled receptor protein signaling pathway, phospholipase C activation,
				positive regulation of cytosolic calcium ion concentration, sensory perception of chemical stimulus
209969_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	2q32.2	regulation of cell cycle, transcription, regulation of transcription, DNA-dependent
				transcription from RNA polymerase II promoter, caspase activation,
				intracellular signaling cascade, I-kappaB kinase/NF-kappaB cascade, tyrosine phosphorylation
				of STAT protein, STAT protein nuclear translocation, response to pest, pathogen or parasite
202388_at	RGS2	regulator of G-protein signalling 2, 24kDa	1q31	cell cycle, signal transduction, regulation of G-protein coupled receptor protein signaling pathway
202626_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	8q13	protein amino acid phosphorylation, intracellular signaling cascade
203104_at	CSF1R	colony stimulating factor 1 receptor, formerly McDonough	5q33-q35	protein amino acid phosphorylation, signal transduction, transmembrane receptor protein tyrosine
		feline sarcoma viral (v-fms) oncogene homolog		kinase signaling pathway, development, cell proliferation, antimicrobial humoral response
204122_at	TYROBP	TYRO protein tyrosine kinase binding protein	19q13.1	cellular defense response, intracellular signaling cascade
209684_at	RIN2	Ras and Rab interactor 2	---	endocytosis, intracellular signaling cascade, small GTPase mediated signal transduction
210222_s_at	RTN1	reticulon 1	14q23.1	signal transduction, neuron cell differentiation
210754_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	8q13	protein amino acid phosphorylation, intracellular signaling cascade
211284_s_at	GRN	granulin	17q21.32	signal transduction, cell-cell signaling, cell proliferation, positive regulation of cell proliferation
216041_x_at	GRN	granulin	17q21.32	signal transduction, cell-cell signaling, cell proliferation, positive regulation of cell proliferation
219607_s_at	MS4A4A	membrane-spanning 4-domains, subfamily A, member 4	11q12	signal transduction
215899_s_at	SCAP2	src family associated phosphoprotein 2	7p21-p15	protein complex assembly, signal transduction
221581_s_at	WBSCR5	Williams-Beuren syndrome chromosome region 5	7q11.23	intracellular signaling cascade, calcium-mediated signaling, B-cell activation
217764_s_at	RAB31	RAB31, member RAS oncogene family	18p11.3	small GTPase mediated signal transduction
217853_at	TENS1	tensin-like SH2 domain containing 1	7p13-p12.3	protein amino acid dephosphorylation, cell cycle, intracellular signaling cascade
218559_s_at	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B	20q11.2-q13.1	transcription, regulation of transcription, DNA-dependent, sensory organ development
204959_at	MNDA	myeloid cell nuclear differentiation antigen	1q22	transcription, regulation of transcription, DNA-dependent, cellular defense response
204039_at	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	19q13.1	generation of precursor metabolites and energy, transcription, regulation of transcription,
				DNA-dependent, transcription from RNA polymerase II promoter
204959_at	MNDA	myeloid cell nuclear differentiation antigen	1q22	transcription, regulation of transcription, DNA-dependent, cellular defense response
205312_at	SPI1	spleen focus forming virus (SFFV) proviral integration oncogene spi1	11p11.2	negative regulation of transcription from RNA polymerase II promoter, transcription,
215933_s_at	HHEX	hematopoietically expressed homeobox	10q23.33	regulation of transcription, DNA-dependent, development, antimicrobial humoral response
				(sensu Vertebrata)
221211_s_at	C21orf7	chromosome 21 open reading frame 7	21q22.3	regulation of transcription, DNA-dependent
205936_s_at	HK3	hexokinase 3 (white cell)	5q35.2	glycolysis
208130_s_at	TBXAS1	thromboxane A synthase 1	7q34-q35	prostaglandin biosynthesis, electron transport, fatty acid biosynthesis, transport, blood coagulation
		(platelet, cytochrome P450, family 5, subfamily A)		
213566_at	RNASE6	ribonuclease, RNase A family, k6	14q11.2	RNA catabolism, defense response
219093_at	FLJ20701	hypothetical protein FLJ20701	2q36.3	---
204619_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
205237_at	FCN1	ficolin (collagen/fibrinogen domain containing) 1	9q34	phosphate transport, cell adhesion, opsonization
201798_s_at	FER1L3	fer-1-like 3, myoferlin (C. elegans)	10q24	muscle contraction, circulation
206133_at	HSXIAPAF1	XIAP associated factor-1	17p13.1	---
201425_at	ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)	12q24.2	carbohydrate metabolism, alcohol metabolism, metabolism
215220_s_at	TPR	translocated promoter region (to activated MET oncogene)	1q25	protein-nucleus import, transport
				protein biosynthesis, tryptophanyl-tRNA aminoacylation, tryptophanyl-tRNA aminoacylation,
201218_at	---	---	---	---
201739_at	SGK	serum/glucocorticoid regulated kinase	6q23	protein amino acid phosphorylation, sodium ion transport, apoptosis, response to stress
202897_at	PTPNS1	protein tyrosine phosphatase, non-receptor type substrate 1	20p13	---
210423_s_at	SLC11A1	solute carrier family 11	2q35	transport, iron ion transport, response to pest, pathogen or parasite, response to bacteria
		(proton-coupled divalent metal ion transporters), member 1		
210873_x_at	APOBEC3A	apolipoprotein B mRNA editing enzyme	22q13.1-q13.2	---
		catalytic polypeptide-like 3A		
215123_at	---	---	---	---
204439_at	IFI44L	interferon-induced protein 44-like	1p31.1	---
204588_s_at	SLC7A7	solute carrier family 7 (cationic amino acid transporter,	14q11.2	protein complex assembly, amino acid metabolism, transport, transport, amino acid transport
		y+ system), member 7		
204619_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
204620_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
204834_at	FGL2	fibrinogen-like 2	7q11.23	---
204249_s_at	LMO2	LIM domain only 2 (rhombotin-like 1)	11p13	development
204971_at	CSTA	cystatin A (stefin A)	3q21	---
205078_s_at	MTMR11	myotubularin related protein 11	1q12-q21	phospholipid dephosphorylation
205237_at	FCN1	ficolin (collagen/fibrinogen domain containing) 1	9q34	phosphate transport, cell adhesion, opsonization
207078_at	MED6	mediator of RNA polymerase II transcription	18p11.32	cortical actin cytoskeleton organization and biogenesis
208148_s_at	CPVL	carboxypeptidase, vitellogenic-like	7p15-p14	proteolysis and peptidolysis
208450_at	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	22q12-q13]22q13.1	---
209683_at	FAM49A	Family with sequence similarity 49, member A	2p24.3-p24.2	---
209949_at	NCF2	neutrophil cytosolic factor 2	1q25	superoxide metabolism, cellular defense response
		(65kDa, chronic granulomatous disease, autosomal 2)		
210663_s_at	KYNU	kynureninase (L-kynurenine hydrolase)	2q22.3	tryptophan catabolism, NAD biosynthesis
211571_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
212192_at	KCTD12	potassium channel tetramerisation domain containing 12	13q22.3	potassium ion transport
212225_at	SUI1	putative translation initiation factor	17q21.2	protein biosynthesis, translational initiation, regulation of translation, regulation of
212636_at	QKI			translational initiation, response to stress
212681_at	EPB41L3	quaking homolog, KH domain RNA binding (mouse)	6q26-27	---
217388_s_at	KYNU	kynureninase (L-kynurenine hydrolase)	2q22.3	tryptophan catabolism, NAD biosynthesis
217679_x_at	---	---	---	---
218035_s_at	FLJ20273	RNA-binding protein	4p13-p12	---
220091_at	SLC2A6	solute carrier family 2 (facilitated glucose transporter), member 6	9q34	carbohydrate transport
220532_s_at	LR8	LR8 protein	7q36.1	organogenesis
221731_x_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
214084_x_at	---	---	---	---
214722_at	NOTCH2NL	Notch homolog 2 (Drosophila) N-terminal like	1q21.2	---
215646_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
216109_at	THRAP2	Thyroid hormone receptor associated protein 2	12q24.21	---

Tab. A1:
Complete list of all probe-sets differentially regulated after IVIG-treatment;
Probe-Sets are selected using following criteria: a minimum of either a 2-fold increase or decrease, in a minimum of 40% (4 out of 10 patients);
Probe-Sets common in 60% of patients are written in purple, probe-sets common in 50% of patients are written in blue;

- Immune-related probe-sets are highlighted in light blue;
- Proliferation-, apoptosis- or cell cyte-related probe-sets are highlighted in grey;
- Signalling-related probe-sets are highlighted in yellow;
- Transcription-related probe-sets are highlighted in green;

Tab. A2

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location	GO Biological Process
206390_x_at	PF4	platelet factor 4 (chemokine (C-X-C motif) ligand 4)	4q12-q21	immune response, sensory perception, negative regulation of angiogenesis,
				cytokine and chemokine mediated signaling pathway, platelet activation, immune cell chemotaxis
				negative regulation of megakaryocyte differentiation
210313_at	ILT7	leukocyte immunoglobulin-like receptor, subfamily A	19q13.4	immune response
		(without TM domain), member 4		
205033_s_at	DEFA1, DEFA3 ↑	defensin, alpha 1, myeloid-related sequence, defensin,	8p23.1, 8pter-p23.3	xenobiotic metabolism, response to pest, pathogen or parasite,
		alpha 3, neutrophil-specific		defense response to bacteria, defense response to fungi
204081_at	NRGN	neurogranin (protein kinase C substrate, RC3)	11q24	signal transduction, neurogenesis
205898_at	CX3CR1	chemokine (C-X3-C motif) receptor 1	3p21|3p21.3	chemotaxis, cellular defense response, cell adhesion, signal transduction,
				G-protein coupled receptor protein signaling pathway
206207_at	CLC	Charcot-Leyden crystal protein	19q13.1	phospholipid metabolism, development, lipid catabolism, antimicrobial humoral response
208450_at	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	22q12-q13|22q13.1	—
212187_x_at	PTGDS	prostaglandin D2 synthase 21kDa (brain)	9q34.2-q34.3	prostaglandin biosynthesis, fatty acid biosynthesis, transport, regulation of circadian sleep/wake cycle
205495_s_at	GNLY	granulysin	2p12-q11	xenobiotic metabolism, cellular defense response, defense response to bacteria and fungi
205898_at	CX3CR1	chemokine (C-X3-C motif) receptor 1	3p21|3p21.3	chemotaxis, cellular defense response, cell adhesion, signal transduction,
				G-protein coupled receptor protein signaling pathway
210164_at	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated	14q11.2	proteolysis and peptidolysis, apoptosis, cleavage of lamin, cytolysis
		serine esterase 1)		
210321_at	GZMH	granzyme H (cathepsin G-like 2, protein h-CCPX)	14q11.2	proteolysis and peptidolysis, apoptosis, cytolysis
213915_at	NKG7	natural killer cell group 7 sequence	19q13.41	—
37145_at	GNLY	granulysin	2p12-q11	xenobiotic metabolism, cellular defense response, defense response to bacteria and fungi

205403_at	IL1R2 ↑	interleukin 1 receptor, type II	2q12-q22	immune response
204006_s_at	FCGR3A/B	Fc fragment of IgG, low affinity IIIa, receptor (CD16a),	1q23	immune response
		Fc fragment of IgG, low affinity IIIb, receptor (CD16b)		
209312_x_at	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	6p21.3	immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
209728_at	HLA-DRB4	major histocompatibility complex, class II, DR beta 4	6p21.3	immune response, signal transduction, pathogenesis, antigen presentation, exogenous antigen,
				antigen processing, exogenous antigen via MHC class II
210982_s_at	HLA-DRA	major histocompatibility complex, class II, DR alpha	6p21.3	immune response, immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
211990_at	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	6p21.3	immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
211991_s_at	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	6p21.3	immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
204006_s_at	FCGR3A/B	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	1q23	immune response
204057_at	IRF8	interferon regulatory factor 8	16q24.1	negative regulation of transcription from RNA polymerase II promoter, transcription,
				regulation of transcription, DNA-dependent, immune response
214181_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis,
				negative regulation of lymphocyte proliferation
215071_s_at	HIST1H2AC	histone 1, H2ac	6p21.3	nucleosome assembly, chromosome organization and biogenesis (sensu Eukaryota)
215193_x_at	HLA-DRB1	major histocompatibility complex, class II, DR beta 1	6p21.3	immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
211991_s_at	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	6p21.3	immune response, antigen presentation, exogenous antigen, antigen processing,
				exogenous antigen via MHC class II
220832_at	TLR8	toll-like receptor 8	xp22	inflammatory response, IkB kinase/NFkB cascade, detection of virus, innate immunity
201506_at	TGFBI	transforming growth factor, beta-induced, 68kDa	5q31	cell adhesion, neg. regulation of cell adhesion, sensory perception, visual perception, cell proliferation
206893_s_at	DUSP6	dual specific phosphatase 6	12q22-q23	regulation of cell cycle, inactivation of MAPK, protein amino acid dephosphorylation
207206_s_at	ALOX12	arachidonate 12-lipoxygenase	17p13.1	electron transport, oxygen and reactive oxygen species metabolism, anti-apoptosis, cell motility
				positive regulation of cell proliferation, positive regulation of cell proliferation, arachidonic acid
				metabolism, leukotriene biosynthesis, fatty acid oxidation, pos. regulation of cell growth, regulation of
				membrane potential, superoxide release, pos. regulation of cell adhesion, neg. regulation of cell volume
214146_s_at	PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	4q12-q13	regulation of cell cycle, chemotaxis, immune response, sensory perception, cell proliferation,
				glucose transport, defense response to bacteria
205488_at	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated	5q11-q12	proteolysis and peptidolysis, apoptosis, cleavage of lamin, immune response, cytolysis
213348_at	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle,
				cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
202388_at	RGS2	regulator of G protein signaling 2, 24kDa	1q31	cell cycle, signal transduction, regulation of G-protein coupled receptor protein signaling pathway
203104_at	CSF1R	colony stimulating factor 1 receptor, formerly McDonough feline	5q33-q35	protein amino acid phosphorylation, signal transduction, transmembrane receptor protein tyrosine
		sarcoma viral (v-fms) oncogene homolog		kinase signaling pathway, development, cell proliferation, antimicrobial humoral response
203485_at	RTN1	reticulon 1	14q23.1	signal transduction, neuron cell differentiation
203680_at	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	7q22	protein amino acid phosphorylation, signal transduction, intracellular signaling cascade
205159_at	CSF2RB	colony stimulating factor 2 receptor, beta,	22q13.1	signal transduction, respiratory gaseous exchange, cytokine and chemokine mediated signaling
		low-affinity (granulocyte-macrophage)		pathway, antimicrobial humoral response (sensu Vertebrata)
206493_at	ITGA2B	integrin, alpha 2b	17q21.32	cell-matrix adhesion, integrin-mediated signaling pathway
		(platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)		
206655_s_at	GP1BB	glycoprotein Ib (platelet), beta polypeptide	22q11.21-q11.23|22q11	cell adhesion, cell surface receptor linked signal transduction, platelet activation
214146_s_at	PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	4q12-q13	regulation of cell cycle, chemotaxis, immune response, sensory perception,
				cell proliferation, glucose transport, defense response to bacteria
203680_at	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	7q22	protein amino acid phosphorylation, signal transduction, intracellular signaling cascade
219529_at	CLIC3	chloride intracellular channel 3	9q34.3	ion transport, chloride transport, signal transduction
214567_s_at	XCL1/XCL2	chemokine (C motif) ligand 1	1q23/1q23-q25	calcium ion homeostasis, chemotaxis, immune response, signal transduction, cell-cell signaling,
				sensory perception, antimicrobial humoral response, chemotaxis, signal transduction,
				cell-cell signaling, circulation
203140_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	3q27	negative regulation of transcription from RNA polymerase II promoter, transcription, regulation of
				transcription, DNA-dependent, inflammatory response, positive regulation of cell proliferation
204057_at	IRF8	interferon regulatory factor 8	16q24.1	negative regulation of transcription from RNA polymerase II promoter, transcription,
				regulation of transcription, DNA-dependent, immune response
204115_at	GNG11	guanine nucleotide binding protein (G protein), gamma 11	7q31-q32	signal transduction, G-protein coupled receptor protein signaling pathway
212331_at	RBL2	retinoblastoma-like 2 (p130)	16q12.2	transcription, regulation of transcription, negative regulation of cell cycle, DNA-dependent cell cycle
221841_s_at	KLF4	Kruppel-like factor 4 (gut)	9q31	transcription, mesoderm cell fate determination, negative regulation of cell proliferation, negative
				regulation of transcription, DNA-dependent, negative regulation of transcription, DNA-dependent
221211_s_at	C21orf7	chromosome 21 open reading frame 7	21q22.3	regulation of transcription, DNA-dependent
205861_at	SPIB	Spi-B transcription factor (Spi-1/PU.1 related)	19q13.3-q13.4	transcription, regulation of transcription from RNA polymerase II promoter
200596_s_at	EIF3S10 ↑	eukaryotic translation initiation factor 3,	10q26	protein biosynthesis, regulation of translational initiation
		subunit 10 theta, 150/170kDa		
200727_s_at	ACTR2 ↑	ARP2 actin-related protein 2 homolog (yeast)	2p14	
203645_s_at	CD163 ↑	CD163 antigen	12p13.3	antimicrobial humoral response (sensu Vertebrata)
204900_x_at	SAP30 ↑	sin3-associated polypeptide, 30kDa	4q34.1	---
212188_at	KCTD12 ↑	potassium channel tetramerisation domain containing 12	13q22.3	potassium ion transport
215049_x_at	CD163 ↑	CD163 antigen	12p13.3	antimicrobial humoral response (sensu Vertebrata)
203645_s_at	CD163 ↑	CD163 antigen	12p13.3	antimicrobial humoral response (sensu Vertebrata)
215646_s_at	CSPG2 ↑	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
200665_s_at	SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	5q31.3-q32	ossification
201669_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	6q22.2	cell motility
203305_at	F13A1	coagulation factor XIII, A1 polypeptide	6p25.3-p24.3	blood coagulation, peptide cross-linking
204439_at	IFI44L	interferon-induced protein 44-like	1p31.1	—
204838_s_at	MLH3	mutL homolog 3 (E. coli)	14q24.3	mismatch repair, meiotic recombination
205495_s_at	GNLY	granulysin	2p12-q11	xenobiotic metabolism, cellular defense response, defense response to bacteria and fungi
206110_at	HIST1H3H	histone 1, H3h	6p22-p21.3	—
208488_s_at	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	7q11.2	lipid metabolism, fatty acid metabolism, transport, cell adhesion, blood coagulation
208146_s_at	CPVL	carboxypeptidase, vitellogenic-like	7p15-p14	proteolysis and peptidolysis
208579_x_at	H2BFS	H2B histone family, member S	21q22.3	nucleosome assembly, chromosome organization and biogenesis (sensu Eukaryota)
208601_s_at	TUBB1	tubulin, beta 1	20q13.32	microtubule-based movement, protein polymerization
208791_at	CLU	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprot.	8p21-p12	lipid metabolism, apoptosis, immune response, complement activation, classical pathway,
		2, testosterone-repressed prostate message 2, apolipoprotein J)		fertilization (sensu Metazoa), cell death
209555_s_at	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)	7q11.2	lipid metabolism, fatty acid metabolism, transport, cell adhesion, blood coagulation
210164_at	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated	14q11.2	proteolysis and peptidolysis, apoptosis, cleavage of lamin, cytolysis
		serine esterase 1)		
211748_x_at	PTGDS	prostaglandin D2 synthase 21kDa (brain)	9q34.2-q34.3	prostaglandin biosynthesis, fatty acid biosynthesis, transport, regulation of circadian sleep/wake cycle
217979_at	TSPAN13	tetraspanin 13	7p21.1	---
219759_at	LRAP	leukocyte-derived arginine aminopeptidase	—	proteolysis and peptidolysis

208450_at	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	22q12-q13|22q13.1	---
211571_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
203799_at	CD302	CD302 antigen	2q24.2	
204960_s_at	BIRC1	baculoviral IAP repeat-containing 1	5q13.1	cell motility
208450_at	LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	22q12-q13|22q13.1	---
211748_x_at	PTGDS	prostaglandin D2 synthase 21kDa (brain)	9q34.2-q34.3	prostaglandin biosynthesis, fatty acid biosynthesis, transport, regulation of circadian sleep/wake cycle
212187_x_at	PTGDS	prostaglandin D2 synthase 21kDa (brain)	9q34.2-q34.3	prostaglandin biosynthesis, fatty acid biosynthesis, transport, regulation of circadian sleep/wake cycle
217979_at	TSPAN13	tetraspanin 13	7p21.1	---

Tab. A2:
Complete list of all probe-sets differentially regulated after IVMP-treatment;
Probe-Sets are selected using following criteria: a minimum of either a 2-fold increase or decrease, in a minimum of 60% (3 out of 5 patients);
Probe-Sets common in 80% of patients are written in purple, probe-sets common in 60% of patients are written in blue;

Immune-related probe-sets are highlighted in light blue;
Proliferation-, apoptosis- or cell cycle-related probe-sets are highlighted in grey;
Signalling-related probe-sets are highlighted in yellow;
Transcription-related probe-sets are highlighted in green;

Tab. A3a

Probe Set ID	log2 (fold change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
219534_x_at	1.999	3,99722837	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
213904_at	1.938	3,83174087	Clone 23555 mRNA sequence	---	---	---
215076_s_at	1.809	3,50399326	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	2q31	phosphate transport /// circulation /// organogenesis
213458_at	1.637	3,11018414	KIAA0974	KIAA0974	10q22.2	---
209169_at	1.589	3,0084075	glycoprotein M6B	GPM6B	Xp22.2	neurogenesis /// cell differentiation
220084_at	1.542	2,9119791	chromosome 14 open reading frame 105	C14orf105	14q22.3	---
220178_at	1.516	2,85996997	chromosome 19 open reading frame 28	C19orf28	19p13.3	---
201616_s_at	1.497	2,82255169	caldesmon 1	CALD1	7q33	muscle contraction /// muscle development
220687_at	1.442	2,71697257	---	---	---	---
221233_s_at	1.438	2,70944995	KIAA1411 /// KIAA1411	KIAA1411	6q12-q13	---
215308_at	1.422	2,6795672	Thyroid autoantigen 70kDa (Ku antigen)	G22P1	22q13.2-q13.31	DNA ligation /// DNA repair /// double-strand break repair via nonhomologous end-joining /// DNA recombination /// positive regulation of transcription, DNA-dependent
210880_s_at	1.416	2,66844634	embryonal Fyn-associated substrate	EFS	14q11.2-q12	cell adhesion /// intracellular signaling cascade
215830_at	1.404	2,64634288	SH3 and multiple ankyrin repeat domains 2	SHANK2	11q13.3-q13.4	intracellular signaling cascade
216814_at	1.395	2,62988552	---	---	---	---
204887_s_at	1.375	2,59367911	polo-like kinase 4 (Drosophila)	PLK4	4q27-q28	regulation of cell cycle /// protein amino acid phosphorylation
215759_at	1.369	2,5829147	hypothetical protein FLJ12056	FLJ12056	2p13.3	---
221183_at	1.354	2,55619873	CDNA: FLJ23604 fis, clone LNG15857	---	---	---
208291_s_at	1.337	2,52625452	tyrosine hydroxylase	TH	11p15.5	synaptic transmission /// aromatic amino acid family metabolism /// morphogenesis /// neurotransmitter biosynthesis /// catecholamine biosynthesis
222305_at	1.311	2,4811346	hexokinase 2	HK2	2p13	regulation of cell cycle /// glycolysis
211448_s_at	1.289	2,4435862	regulator of G-protein signalling 6	RGS6	14q24.3	G-protein coupled receptor protein signaling pathway /// intracellular signaling cascade /// regulation of G-protein coupled receptor protein signaling pathway
206446_s_at	1.275	2,41998818	elastase 2A	ELA2A	1p36.21	proteolysis and peptidolysis
206903_at	1.274	2,41831135	---	---	---	---
220411_x_at	1.267	2,40660605	hypothetical protein FLJ23447	FLJ23447	19p13.12	---
215070_x_at	1.263	2,39994276	RAB GTPase activating protein 1	RABGAP1	9q33.2-q33.3	cell cycle
214099_s_at	1.256	2,38832637	phosphodiesterase 4D interacting protein (myomegalin)	PDE4DIP	1q12	protein biosynthesis /// cytoskeleton organization and biogenesis /// actin cytoskeleton organization and biogenesis
210801_at	1.237	2,35707882	dimethyladenosine transferase	HSA9761	5q11-q14	rRNA modification /// rRNA processing
208153_s_at	1.231	2,34729636	FAT tumor suppressor homolog 2 (Drosophila) /// FAT tumor suppressor homolog 2 (Drosophila)	FAT2	5q32-q33	cell adhesion /// homophilic cell adhesion
217518_at	1.221	2,3310824	fer-1-like 3, myoferlin (C. elegans)	FER1L3	10q24	muscle contraction /// circulation
215211_at	1.217	2,32462822	Clone 23832 mRNA sequence	---	---	---
209866_s_at	1.198	2,29421405	latrophilin 3	LPHN3	4q13.1	signal transduction /// neuropeptide signaling pathway

220382_s_at	1.186	2,27521046	Rho GTPase activating protein 28	ARHGAP28	18p11.23	viral release
216856_s_at	1.162	2,2376742	---	---	---	---
214133_at	1.148	2,2160647	mucin 6, gastric	MUC6	11p15.5-p15.4	---
217685_at	1.145	2,21146131	Solute carrier family 16 (monocarboxylic acid transporters), member 3	SLC16A3	17q25	transport /// organic anion transport /// monocarboxylic acid transport
213516_at	1.145	2,21146131	A kinase (PRKA) anchor protein 13	AKAP13	15q24-q25	intracellular signaling cascade
211154_at	1.129	2,18707091	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	THPO	3q27	development /// cell proliferation
207662_at	1.128	2,18555548	T-box 1	TBX1	22q11.21	transcription /// regulation of transcription from RNA polymerase II promoter /// heart development /// morphogenesis
209819_at	1.119	2,17196371	hyaluronan binding protein 4	HABP4	9q22.3-q31	---
209969_s_at	1.119	2,17196371	signal transducer and activator of transcription 1, 91kDa	STAT1	2q32.2	regulation of cell cycle /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// caspase activation /// intracellular signaling cascade /// I-kappaB kinase/NF-kappaB cascade /// tyrosine phosp
210019_at	1.113	2,16294953	calmodulin-like 3	CALML3	10pter-p13	---
205249_at	1.095	2,13613082	early growth response 2 (Krox-20 homolog, Drosophila)	EGR2	10q21.1	transcription /// regulation of transcription, DNA-dependent /// brain development /// peripheral nervous system development /// mechanosensory behavior
218978_s_at	1.089	2,12726535	mitochondrial solute carrier protein	MSCP	8p21.2	transport
216165_at	1.086	2,12284642	CDNA: FLJ21997 fis, clone HEP06590	---	---	---
204257_at	1.065	2,09216988	fatty acid desaturase 3	FADS3	11q12-q13.1	fatty acid biosynthesis /// fatty acid desaturation /// fatty acid desaturation
217363_x_at	1.048	2,06766147	---	---	---	---
215013_s_at	1.035	2,04911365	ubiquitin specific protease 34	USP34	2p15	ubiquitin-dependent protein catabolism /// ubiquitin cycle
215942_s_at	1.033	2,04627494	G-2 and S-phase expressed 1	GTSE1	22q13.2-q13.3	G2 phase of mitotic cell cycle /// DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest /// microtubule-based process
204816_s_at	1.027	2,03778239	DEAH (Asp-Glu-Ala-His) box polypeptide 34	DHX34	19q13.3	electron transport
217395_at	1.023	2,03214029	metallothionein IV	MT4	16q13	---
207557_s_at	1.022	2,0307322	ryanodine receptor 2 (cardiac)	RYR2	1q42.1-q43	cation transport /// calcium ion transport /// calcium ion homeostasis /// muscle contraction /// signal transduction /// regulation of heart contraction rate
204677_at	1.015	2,02090289	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
206926_s_at	1.003	2,00416321	interleukin 11	IL11	19q13.3-q13.4	cell-cell signaling /// positive regulation of cell proliferation /// platelet activation /// B-cell differentiation /// megakaryocyte differentiation /// adipocyte differentiation

Tab. A3a:
Probe-sets up-regulated regulated upon IVIG treatment at day 6 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A3a

Probe Set ID	log2 (fold change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
219534_x_at	1.999	3,99722837	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
213904_at	1.938	3,83174087	Clone 23555 mRNA sequence	---	---	---
215076_s_at	1.809	3,50399326	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	2q31	phosphate transport /// circulation /// organogenesis
213458_at	1.637	3,11018414	KIAA0974	KIAA0974	10q22.2	---
209169_at	1.589	3,0084075	glycoprotein M6B	GPM6B	Xp22.2	neurogenesis /// cell differentiation
220084_at	1.542	2,9119791	chromosome 14 open reading frame 105	C14orf105	14q22.3	---
220178_at	1.516	2,85996997	chromosome 19 open reading frame 28	C19orf28	19p13.3	---
201616_s_at	1.497	2,82255169	caldesmon 1	CALD1	7q33	muscle contraction /// muscle development
220687_at	1.442	2,71697257	---	---	---	---
221233_s_at	1.438	2,70944995	KIAA1411 /// KIAA1411	KIAA1411	6q12-q13	---
215308_at	1.422	2,6795672	Thyroid autoantigen 70kDa (Ku antigen)	G22P1	22q13.2-q13.31	DNA ligation /// DNA repair /// double-strand break repair via nonhomologous end-joining /// DNA recombination /// positive regulation of transcription, DNA-dependent
210880_s_at	1.416	2,66844634	embryonal Fyn-associated substrate	EFS	14q11.2-q12	cell adhesion /// intracellular signaling cascade
215830_at	1.404	2,64634288	SH3 and multiple ankyrin repeat domains 2	SHANK2	11q13.3-q13.4	intracellular signaling cascade
216814_at	1.395	2,62988552	---	---	---	---
204887_s_at	1.375	2,59367911	polo-like kinase 4 (Drosophila)	PLK4	4q27-q28	regulation of cell cycle /// protein amino acid phosphorylation
215759_at	1.369	2,5829147	hypothetical protein FLJ12056	FLJ12056	2p13.3	---
221183_at	1.354	2,55619873	CDNA: FLJ23604 fis, clone LNG15857	---	---	---
208291_s_at	1.337	2,52625452	tyrosine hydroxylase	TH	11p15.5	synaptic transmission /// aromatic amino acid family metabolism /// morphogenesis /// neurotransmitter biosynthesis /// catecholamine biosynthesis
222305_at	1.311	2,4811346	hexokinase 2	HK2	2p13	regulation of cell cycle /// glycolysis
211448_s_at	1.289	2,4435862	regulator of G-protein signalling 6	RGS6	14q24.3	G-protein coupled receptor protein signaling pathway /// intracellular signaling cascade /// regulation of G-protein coupled receptor protein signaling pathway
206446_s_at	1.275	2,41998818	elastase 2A	ELA2A	1p36.21	proteolysis and peptidolysis
206903_at	1.274	2,41831135	---	---	---	---
220411_x_at	1.267	2,40660605	hypothetical protein FLJ23447	FLJ23447	19p13.12	---
215070_x_at	1.263	2,39994276	RAB GTPase activating protein 1	RABGAP1	9q33.2-q33.3	cell cycle
214099_s_at	1.256	2,38832637	phosphodiesterase 4D interacting protein (myomegalin)	PDE4DIP	1q12	protein biosynthesis /// cytoskeleton organization and biogenesis /// actin cytoskeleton organization and biogenesis
210801_at	1.237	2,35707882	dimethyladenosine transferase	HSA9761	5q11-q14	rRNA modification /// rRNA processing
208153_s_at	1.231	2,34729636	FAT tumor suppressor homolog 2 (Drosophila) /// FAT tumor suppressor homolog 2 (Drosophila)	FAT2	5q32-q33	cell adhesion /// homophilic cell adhesion
217518_at	1.221	2,3310824	fer-1-like 3, myoferlin (C. elegans)	FER1L3	10q24	muscle contraction /// circulation
215211_at	1.217	2,32462822	Clone 23832 mRNA sequence	---	---	---
209866_s_at	1.198	2,29421405	latrophilin 3	LPHN3	4q13.1	signal transduction /// neuropeptide signaling pathway

220382_s_at	1.186	2,27521046	Rho GTPase activating protein 28	ARHGAP28	18p11.23	viral release
216856_s_at	1.162	2,2376742	---	---	---	---
214133_at	1.148	2,2160647	mucin 6, gastric	MUC6	11p15.5-p15.4	---
217685_at	1.145	2,21146131	Solute carrier family 16 (monocarboxylic acid transporters), member 3	SLC16A3	17q25	transport /// organic anion transport /// monocarboxylic acid transport
213516_at	1.145	2,21146131	A kinase (PRKA) anchor protein 13	AKAP13	15q24-q25	intracellular signaling cascade
211154_at	1.129	2,18707091	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	THPO	3q27	development /// cell proliferation
207662_at	1.128	2,18555548	T-box 1	TBX1	22q11.21	transcription /// regulation of transcription from RNA polymerase II promoter /// heart development /// morphogenesis
209819_at	1.119	2,17196371	hyaluronan binding protein 4	HABP4	9q22.3-q31	---
209969_s_at	1.119	2,17196371	signal transducer and activator of transcription 1, 91kDa	STAT1	2q32.2	regulation of cell cycle /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// caspase activation /// intracellular signaling cascade /// I-kappaB kinase/NF-kappaB cascade /// tyrosine phosp
210019_at	1.113	2,16294953	calmodulin-like 3	CALML3	10pter-p13	---
205249_at	1.095	2,13613082	early growth response 2 (Krox-20 homolog, Drosophila)	EGR2	10q21.1	transcription /// regulation of transcription, DNA-dependent /// brain development /// peripheral nervous system development /// mechanosensory behavior
218978_s_at	1.089	2,12726535	mitochondrial solute carrier protein	MSCP	8p21.2	transport
216165_at	1.086	2,12284642	CDNA: FLJ21997 fis, clone HEP06590	---	---	---
204257_at	1.065	2,09216988	fatty acid desaturase 3	FADS3	11q12-q13.1	fatty acid biosynthesis /// fatty acid desaturation /// fatty acid desaturation
217363_x_at	1.048	2,06766147	---	---	---	---
215013_s_at	1.035	2,04911365	ubiquitin specific protease 34	USP34	2p15	ubiquitin-dependent protein catabolism /// ubiquitin cycle
215942_s_at	1.033	2,04627494	G-2 and S-phase expressed 1	GTSE1	22q13.2-q13.3	G2 phase of mitotic cell cycle /// DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest /// microtubule-based process
204816_s_at	1.027	2,03778239	DEAH (Asp-Glu-Ala-His) box polypeptide 34	DHX34	19q13.3	electron transport
217395_at	1.023	2,03214029	metallothionein IV	MT4	16q13	---
207557_s_at	1.022	2,0307322	ryanodine receptor 2 (cardiac)	RYR2	1q42.1-q43	cation transport /// calcium ion transport /// calcium ion homeostasis /// muscle contraction /// signal transduction /// regulation of heart contraction rate
204677_at	1.015	2,02090289	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
206926_s_at	1.003	2,00416321	interleukin 11	IL11	19q13.3-q13.4	cell-cell signaling /// positive regulation of cell proliferation /// platelet activation /// B-cell differentiation /// megakaryocyte differentiation /// adipocyte differentiation

Tab. A3a:
Probe-sets up-regulated regulated upon IVIG treatment at day 6 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab.A3b

Probe Set ID	log2 (fold Change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
215793_at	-2.268	-4,81654952	myotubularin related protein 7	MTMR7	8p22	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// cell cycle /// phospholipid dephosphorylation
205122_at	-1.985	-3,95862663	transmembrane protein with EGF-like and two follistatin-like domains 1	TMEFF1	9q31	---
213258_at	-1.64	-3,11665832	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	TFPI	2q31-q32.1	blood coagulation
209615_s_at	-1.494	-2,81668845	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	PAK1	11q13-q14	protein amino acid phosphorylation /// protein amino acid phosphorylation /// apoptosis /// JNK cascade
209612_s_at	-1.403	-2,64450921	alcohol dehydrogenase IB (class I), beta polypeptide	ADH1B	4q21-q23	ethanol oxidation
201430_s_at	-1.403	-2,64450921	dihydropyrimidinase-like 3	DPYSL3	5q32	nucleobase, nucleoside, nucleotide and nucleic acid metabolism /// signal transduction /// neurogenesis
222135_at	-1.398	-2,6353599	Similar to Hypothetical zinc finger protein KIAA1956	---	19q13.43	---
204591_at	-1.329	-2,51228476	cell adhesion molecule with homology to L1CAM (close homolog of L1)	CHL1	3p26.1	cell adhesion /// signal transduction
220258_s_at	-1.328	-2,51054398	hypothetical protein FLJ10385	FLJ10385	17p13.1	---
217082_at	-1.288	-2,44189303	Unknown protein	---	---	---
211831_s_at	-1.286	-2,43851019	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	THPO	3q27	development /// cell proliferation
214160_at	-1.271	-2,41328784	---	---	---	---
217386_at	-1.244	-2,36854322	---	---	---	---
215381_at	-1.228	-2,34242036	FK506 binding protein 12-rapamycin associated protein 1	FRAP1	1p36.2	regulation of cell cycle /// DNA repair /// DNA recombination
214929_s_at	-1.223	-2,3343162	KIAA1109	KIAA1109	4q27	---
211480_s_at	-1.206	-2,30697121	solute carrier organic anion transporter family, member 1A2	SLCO1A2	12p12	transport /// ion transport /// organic anion transport
210702_s_at	-1.186	-2,27521046	prostaglandin I2 (prostacyclin) synthase	PTGIS	20q13.13	prostaglandin biosynthesis /// electron transport /// lipid metabolism /// fatty acid biosynthesis
216596_at	-1.178	-2,26262893	similar to DKFZP434L187 protein	LOC440265	15q13.3	---
216225_at	-1.161	-2,2361237	Anthrax toxin receptor 1	ANTXR1	2p13.1	---
214381_at	-1.16	-2,23457428	similar to CDC10 (cell division cycle 10, S.cerevisiae, homolog)	LOC441601	11p11.12	cell cycle
222202_at	-1.158	-2,23147864	CDNA FLJ14293 fis, clone PLACE1007866	---	---	---
208028_s_at	-1.155	-2,22684324	glutathione peroxidase 5 (epididymal androgen-related protein)	GPX5	6p22.1	lipid metabolism /// response to oxidative stress
206519_x_at	-1.144	-2,20992897	---	---	---	---
210429_at	-1.127	-2,18404109	Rhesus blood group, D antigen	RHD	1p36.11	---
208514_at	-1.126	-2,18252775	potassium voltage-gated channel, Isk-related family, member 1	KCNE1	21q22.1-q22.2|21q22.12	ion transport /// potassium ion transport /// muscle contraction /// perception of sound /// regulation of heart contraction rate
209590_at	-1.116	-2,16745193	Bone morphogenetic protein 7 (osteogenic protein 1)	BMP7	20q13	skeletal development /// cell differentiation /// growth
220180_at	-1.097	-2,13909418	CTCL tumor antigen se57-1	SE57-1	18q21	---

209465_x_at	-1.093	-2,13317156	pleiotrophin (heparin binding growth factor 8, neurite growth promoting factor 1)	PTN	7q33-q34	regulation of cell cycle /// transmembrane receptor protein tyrosine phosphatase signaling pathway /// neurogenesis /// cell proliferation /// positive regulation of cell proliferation
203862_s_at	-1.083	-2,11843667	actinin, alpha 2	ACTN2	1q42-q43	---
214588_s_at	-1.081	-2,11550193	Microfibrillar-associated protein 3	MFAP3	5q32-q33.2	---
221922_at	-1.065	-2,09216988	G-protein signalling modulator 2 (AGS3-like, C. elegans)	GPSM2	1p13.3	signal transduction /// G-protein coupled receptor protein signaling pathway
214803_at	-1.055	-2,07771821	CDNA clone IMAGE:4152983, partial cds	---	---	---
216896_at	-1.045	-2,06336636	collagen, type IV, alpha 3 (Goodpasture antigen)	COL4A3	2q36-q37	proteolysis and peptidolysis /// phosphate transport /// induction of apoptosis /// caspase activation /// cell adhesion /// cell surface receptor linked signal transduction /// perception of sound /// circulation /// cell proliferation /// negative regul
222074_at	-1.028	-2,03919537	uroporphyrinogen decarboxylase	UROD	1p34	heme biosynthesis
205906_at	-1.028	-2,03919537	forkhead box J1	FOXJ1	17q22-17q25	transcription /// regulation of transcription, DNA-dependent /// spermatogenesis
221681_s_at	-1.019	-2,0265138	dentin sialophosphoprotein	DSPP	4q21.3	ossification /// cell adhesion /// development /// perception of sound
204531_s_at	-1.01	-2,0139111	breast cancer 1, early onset	BRCA1	17q21	cell cycle checkpoint /// DNA repair /// regulation of transcription from RNA polymerase II promoter /// regulation of transcription from RNA polymerase III promoter /// DNA damage response, signal transduction by p53 class mediator resulting in transcrip
204414_at	-1.004	-2,00555287	---	---	---	---
217688_at	-1.002	-2,00277451	---	---	---	---

Tab. A3b:
Probe-sets down-regulated regulated upon IVIG treatment at day 6 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A3c

Probe Set ID	log2 (fold Change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
220178_at	2.091	4,26043281	chromosome 19 open reading frame 28	C19orf28	19p13.3	---
217452_s_at	1.768	3,40581483	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	B3GALT2	1q31	protein amino acid glycosylation
215554_at	1.738	3,33572418	glycosylphosphatidylinositol specific phospholipase D1	GPLD1	6p22.3-p22.2	cell-matrix adhesion
204816_s_at	1.607	3,04617748	DEAH (Asp-Glu-Ala-His) box polypeptide 34	DHX34	19q13.3	electron transport
215200_x_at	1.572	2,97316597	Villin 2 (ezrin)	VIL2	6q25.2-q26	cellular morphogenesis /// cytoskeletal anchoring
206863_x_at	1.556	2,94037467	---	---	---	---
220084_at	1.514	2,85600796	chromosome 14 open reading frame 105	C14orf105	14q22.3	---
214676_x_at	1.5	2,82842712	mucin 3B	MUC3B	7q22	---
213904_at	1.498	2,82450881	Clone 23555 mRNA sequence	---	---	---
208580_x_at	1.447	2,72640521	histone 1, H4k /// histone 1, H4j	HIST1H4K /// HIST1H4J	6p22-p21.3	---
214126_at	1.404	2,64634288	Mitochondrial carrier triple repeat 1	MCART1	9p13.3-p12	transport
217539_at	1.402	2,64267681	chromosome 18 open reading frame 25	C18orf25	18q21.1	---
202563_at	1.38	2,60268371	chromosome 14 open reading frame 1	C14orf1	14q24.3	sterol biosynthesis
221233_s_at	1.369	2,5829147	KIAA1411 /// KIAA1411	KIAA1411	6q12-q13	---
220077_at	1.353	2,55442752	hypothetical protein FLJ22349	FLJ22349	22q13.2	---
208546_x_at	1.343	2,5367828	histone 1, H2bh	HIST1H2BH	6p21.3	nucleosome assembly /// nucleosome assembly /// chromosome organization and biogenesis (sensu Eukaryota)
220411_x_at	1.336	2,52450406	hypothetical protein FLJ23447	FLJ23447	19p13.12	---
203806_s_at	1.335	2,52275482	Fanconi anemia, complementation group A /// Fanconi anemia, complementation group A	FANCA	16q24.3	DNA repair /// protein complex assembly
216740_at	1.327	2,50880441	Transcriptional regulating factor 1	TRERF1	6p21.1-p12.1	regulation of transcription, DNA-dependent /// steroid biosynthesis /// cholesterol catabolism /// development /// homeostasis /// positive regulation of transcription, DNA-dependent /// regulation of hormone biosynthesis
214133_at	1.325	2,50532888	mucin 6, gastric	MUC6	11p15.5-p15.4	---
217588_at	1.323	2,50185816	cation channel, sperm associated 2	CATSPER2	15q14	cation transport
206423_at	1.307	2,47426496	angiopoietin-like 7	ANGPTL7	1p36.3-p36.2	response to oxidative stress
213516_at	1.299	2,46058269	A kinase (PRKA) anchor protein 13	AKAP13	15q24-q25	intracellular signaling cascade
205649_s_at	1.291	2,44697608	fibrinogen, A alpha polypeptide	FGA	4q28	blood coagulation /// regulation of blood pressure /// positive regulation of cell proliferation
208061_at	1.29	2,44528056	---	---	---	---
213999_at	1.283	2,43344472	Hypothetical protein MGC11061	MGC11061	2p22.3	---
222305_at	1.277	2,42334532	hexokinase 2	HK2	2p13	regulation of cell cycle /// glycolysis
210880_s_at	1.271	2,41328784	embryonal Fyn-associated substrate	EFS	14q11.2-q12	cell adhesion /// intracellular signaling cascade
215618_at	1.27	2,41161566	Ras suppressor protein 1	RSU1	10p13	signal transduction
217363_x_at	1.27	2,41161566	---	---	---	---

202837_at	1.258	2,39163959	FLN29 gene product	FLN29	12q	---
206366_x_at	1.252	2,3817137	chemokine (C motif) ligand 2	XCL2	1q23-q25	chemotaxis /// immune response /// signal transduction /// cell-cell signaling /// sensory perception /// circulation
204936_at	1.232	2,34892394	mitogen-activated protein kinase kinase kinase kinase 2	MAP4K2	11q13	protein amino acid phosphorylation /// vesicle targeting /// response to stress /// immune response /// protein kinase cascade /// JNK cascade /// hemocyte development
205223_at	1.227	2,34079728	DEP domain containing 5	DEPDC5	22q12.3	intracellular signaling cascade
212478_at	1.226	2,33917533	hypothetical protein FLJ13910	FLJ13910	2p11.2	---
215759_at	1.219	2,32785307	hypothetical protein FLJ12056	FLJ12056	2p13.3	---
220938_s_at	1.211	2,31498043	glucocorticoid modulatory element binding protein 1	GMEB1	1p35.3	---
214692_s_at	1.208	2,31017157	jerky homolog (mouse)	JRK	8q24.3	---
204896_s_at	1.191	2,28310941	prostaglandin E receptor 4 (subtype EP4)	PTGER4	5p13.1	immune response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein signaling, coupled to cAMP nucleotide second messenger
203759_at	1.189	2,27994655	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	ST3GAL4	11q23-q24	protein amino acid glycosylation
210066_s_at	1.16	2,23457428	aquaporin 4	AQP4	18q11.2-q12.1	transport /// neurogenesis /// excretion
215942_s_at	1.153	2,22375832	G-2 and S-phase expressed 1	GTSE1	22q13.2-q13.3	G2 phase of mitotic cell cycle /// DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest /// microtubule-based process
210960_at	1.136	2,19770844	adrenergic, alpha-1D-, receptor	ADRA1D	20p13	DNA metabolism /// signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein signaling, coupled to cAMP nucleotide second messenger /// cell-cell signaling /// development /// cell proliferation /// positive regulation of
204643_s_at	1.133	2,19314318	cytosolic ovarian carcinoma antigen 1	COVA1	Xq25-q26.2	regulation of cell growth /// electron transport /// transport /// ultradian rhythm
205056_s_at	1.129	2,18707091	gene rich cluster, A gene	GRCA	12p13	G-protein coupled receptor protein signaling pathway /// protein metabolism
220420_at	1.125	2,18101547	lectin, mannose-binding, 1 like	LMAN1L	15q24.1	---
204811_s_at	1.117	2,16895482	calcium channel, voltage-dependent, alpha 2/delta subunit 2	CACNA2D2	3p21.3	ion transport /// calcium ion transport
204833_at	1.113	2,16294953	APG12 autophagy 12-like (S. cerevisiae)	APG12L	5q21-q22	autophagic vacuole formation /// ubiquitin cycle /// autophagy /// apoptosis
207557_s_at	1.109	2,15696086	ryanodine receptor 2 (cardiac)	RYR2	1q42.1-q43	cation transport /// calcium ion transport /// calcium ion homeostasis /// muscle contraction /// signal transduction /// regulation of heart contraction rate
211259_s_at	1.093	2,13317156	bone morphogenetic protein 7 (osteogenic protein 1)	BMP7	20q13	skeletal development /// cell differentiation /// growth
208324_at	1.087	2,12431837	---	---	---	---
209299_x_at	1.086	2,12284642	peptidylprolyl isomerase (cyclophilin)-like 2	PPIL2	22q11.21	protein folding

216258_s_at	1.065	2,09216988	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 13	SERPINB13	18q21.3-q22	response to UV /// regulation of proteolysis and peptidolysis
205024_s_at	1.065	2,09216988	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	RAD51	15q15.1	double-strand break repair via homologous recombination /// double-strand break repair via homologous recombination /// DNA unwinding /// DNA unwinding /// DNA repair /// mitotic recombination /// meiosis /// meiotic recombination /// positive regulation
214123_s_at	1.058	2,0820432	chromosome 4 open reading frame 10	C4orf10	4p16.3	---
215402_at	1.055	2,07771821	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	APPBP2	17q21-q23	intracellular protein transport
204482_at	1.054	2,07627854	claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)	CLDN5	22q11.21	calcium-independent cell-cell adhesion
215624_at	1.051	2,07196553	Tuberous sclerosis 2	TSC2	16p13.3	protein folding /// endocytosis /// negative regulation of cell cycle
215409_at	1.05	2,07052985	PLSC domain containing protein	LOC254531	15q14	metabolism
215830_at	1.05	2,07052985	SH3 and multiple ankyrin repeat domains 2	SHANK2	11q13.3-q13.4	intracellular signaling cascade
214530_x_at	1.047	2,06622878	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	EPB41	1p33-p32	circulation /// cortical actin cytoskeleton organization and biogenesis
220185_at	1.034	2,0476938	spectrin, beta, non-erythrocytic 4	SPTBN4	19q13.13	cytoskeletal anchoring /// cytoskeletal anchoring /// vesicle-mediated transport /// vesicle-mediated transport
222258_s_at	1.032	2,04485706	SH3-domain binding protein 4	SH3BP4	2q37.1-q37.2	endocytosis /// cell cycle
206595_at	1.028	2,03919537	cystatin E/M	CST6	11q13	morphogenesis
219203_at	1.027	2,03778239	chromosome 14 open reading frame 122	C14orf122	14q11.2	---
204056_s_at	1.025	2,03495938	mevalonate kinase (mevalonic aciduria)	MVK	12q24	protein amino acid phosphorylation /// cholesterol biosynthesis /// isoprenoid biosynthesis
208906_at	1.019	2,0265138	Bernardinelli-Seip congenital lipodystrophy 2 (seipin) /// hypothetical protein DKFZp762N1910	BSCL2 /// DKFZp762N1910	11q12-q13.5 /// 11q12.3	---
211140_s_at	1.017	2,0237064	caspase 2, apoptosis-related cysteine protease (neural precursor cell expressed, developmentally down-regulated 2)	CASP2	7q34-q35	proteolysis and peptidolysis /// proteolysis and peptidolysis /// anti-apoptosis /// apoptotic program /// regulation of apoptosis
214095_at	1.013	2,01810327	serine hydroxymethyltransferase 2 (mitochondrial)	SHMT2	12q12-q14	glycine metabolism /// L-serine metabolism /// one-carbon compound metabolism
206175_x_at	1.009	2,01251565	zinc finger protein 222	ZNF222	19q13.2	transcription /// regulation of transcription, DNA-dependent
211792_s_at	1.003	2,00416321	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	CDKN2C	1p32	cell cycle /// cell cycle arrest /// negative regulation of cell proliferation

Tab. A3c:
Probe-sets up-regulated regulated upon IVIG treatment at day 21 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A3d

Probe Set ID	log2 (fold change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
215850_s_at	-1.962	-3,89601707	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	NDUFA5	7q32	electron transport
207852_at	-1.741	-3,34266784	chemokine (C-X-C motif) ligand 5	CXCL5	4q12-q13	chemotaxis /// inflammatory response /// signal transduction /// cell-cell signaling /// positive regulation of cell proliferation
219780_at	-1.739	-3,33803712	mesenchymal stem cell protein DSC43	LOC51333	16p11.2	---
211334_at	-1.636	-3,10802908	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	MRE11A	11q21	regulation of mitotic recombination /// double-strand break repair via nonhomologous end-joining /// telomerase-dependent telomere maintenance /// meiosis /// meiotic recombination
213712_at	-1.592	-3,01466982	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2	ELOVL2	6p24.1	fatty acid biosynthesis
216003_at	-1.519	-2,8659233	CMT1A duplicated region transcript 1	CDRT1	17p12	---
219789_at	-1.511	-2,85007523	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	NPR3	5p14-p13	skeletal development
216948_at	-1.483	-2,79529394	---	---	---	---
219312_s_at	-1.464	-2,75872184	zinc finger and BTB domain containing 10	ZBTB10	8q13-q21.1	transcription /// regulation of transcription, DNA-dependent
215793_at	-1.458	-2,74727247	myotubularin related protein 7	MTMR7	8p22	protein amino acid dephosphorylation /// protein amino acid dephosphorylation /// cell cycle /// phospholipid dephosphorylation
214716_at	-1.452	-2,73587061	BMP2 inducible kinase	BMP2K	4q21.21	protein amino acid phosphorylation
211627_x_at	-1.442	-2,71697257	estrogen receptor 1 /// estrogen receptor 1	ESR1	6q25.1	regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// signal transduction /// cell growth /// estrogen receptor signaling pathway /// negative regulation of mitosis
205151_s_at	-1.441	-2,71508996	KIAA0644 gene product	KIAA0644	7p15.1	---
207462_at	-1.44	-2,71320865	glycine receptor, alpha 2	GLRA2	Xp22.1-p21.3	ion transport /// chloride transport /// cell surface receptor linked signal transduction /// synaptic transmission
222246_at	-1.427	-2,68886999	Syntaxin binding protein 3	STXBP3	1p13.3	vesicle docking during exocytosis /// protein transport /// vesicle-mediated transport
205028_at	-1.405	-2,64817782	trophinin	TRO	Xp11.22-p11.21	cell adhesion /// homophilic cell adhesion /// embryo implantation
221136_at	-1.381	-2,60448838	growth differentiation factor 2	GDF2	10q11.22	growth
211238_at	-1.371	-2,58649786	a disintegrin and metalloproteinase domain 7	ADAM7	8p21.2	proteolysis and peptidolysis
210553_x_at	-1.356	-2,55974483	proprotein convertase subtilisin/kexin type 6	PCSK6	15q26.3	proteolysis and peptidolysis /// cell-cell signaling
206294_at	-1.343	-2,5367828	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	HSD3B2	1p13.1	C21-steroid hormone biosynthesis
216634_at	-1.336	-2,52450406	phospholipase C-like 3	PLCL3	3q25.31	lipid metabolism /// intracellular signaling cascade
216419_at	-1.329	-2,51228476	ciliary rootlet coiled-coil, rootletin	CROCC	1pter-p36.11	---
206344_at	-1.325	-2,50532888	paraoxonase 1	PON1	7q21.3	response to external stimulus
208514_at	-1.324	-2,50359292	potassium voltage-gated channel, Isk-related family, member 1	KCNE1	21q22.1-q22.2|21q22.12	ion transport /// potassium ion transport /// muscle contraction /// perception of sound /// regulation of heart contraction rate
207850_at	-1.297	-2,45717396	chemokine (C-X-C motif) ligand 3	CXCL3	4q21	chemotaxis /// inflammatory response /// G-protein coupled receptor protein signaling pathway /// sensory perception
206552_s_at	-1.296	-2,45547137	tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K, neuropeptide gamma)	TAC1	7q21-q22	tachykinin signaling pathway /// neuropeptide signaling pathway /// cell-cell signaling /// synaptic transmission /// insemination /// detection of abiotic stimulus
209465_x_at	-1.281	-2,43007358	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	PTN	7q33-q34	regulation of cell cycle /// transmembrane receptor protein tyrosine phosphatase signaling pathway /// neurogenesis /// cell proliferation /// positive regulation of cell proliferation
205317_s_at	-1.268	-2,40827476	solute carrier family 15 (H+/peptide transporter), member 2	SLC15A2	3q13.33	transport /// oligopeptide transport
207333_at	-1.259	-2,39329793	neuromedin B receptor	NMBR	6q21-qter	signal transduction /// G-protein signaling, coupled to IP3 second messenger (phospholipase C activating)
210429_at	-1.255	-2,38667149	Rhesus blood group, D antigen	RHD	1p36.11	---
219984_s_at	-1.236	-2,35544558	HRAS-like suppressor	HRASLS	3q29	---
208033_s_at	-1.231	-2,34729636	AT-binding transcription factor 1	ATBF1	16q22.3-q23.1	regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter
222135_at	-1.228	-2,34242036	Similar to Hypothetical zinc finger protein KIAA1956	---	19q13.43	---
220180_at	-1.218	-2,32624008	CTCL tumor antigen se57-1	SE57-1	18q21	---
201124_at	-1.216	-2,32301746	integrin, beta 5	ITGB5	3q21.2	cell-matrix adhesion /// integrin-mediated signaling pathway /// development
207174_at	-1.214	-2,31979931	glypican 5	GPC5	13q32	---
211353_at	-1.213	-2,3181919	leucine rich repeat containing 21	LRRC21	10q23	---
214451_at	-1.212	-2,31658561	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	TFAP2B	6p21-p12	transcription /// regulation of transcription from RNA polymerase II promoter /// neurogenesis
207201_s_at	-1 208	-2,31017157	solute carrier family 22 (organic cation transporter), member 1	SLC22A1	6q26	ion transport /// sodium ion transport /// organic cation transport

47550_at	-1.204	-2,30377528	leucine zipper, putative tumor suppressor 1	LZTS1	8p22	transcription /// regulation of transcription, DNA-dependent /// cell cycle /// negative regulation of cell cycle
216577_at	-1.194	-2,28786195	---	---	---	---
219859_at	-1.174	-2,25636427	C-type lectin domain family 4, member E	CLEC4E	12p13.31	immune response /// antimicrobial humoral response (sensu Vertebrata)
216225_at	-1.166	-2,24388696	Anthrax toxin receptor 1	ANTXR1	2p13.1	---
217504_at	-1.159	-2,23302592	ATP-binding cassette, sub-family A (ABC1), member 6	ABCA6	17q24.3	transport
205360_at	-1.158	-2,23147864	prefoldin 4	PFDN4	20q13.2	protein folding /// chaperonin-mediated tubulin folding
208028_s_at	-1.158	-2,23147864	glutathione peroxidase 5 (epididymal androgen-related protein)	GPX5	6p22.1	lipid metabolism /// response to oxidative stress
205517_at	-1.144	-2,20992897	GATA binding protein 4	GATA4	8p23.1-p22	transcription /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// development /// heart development /// positive regulation of transcription, DNA-dependent /// positive regulation of trans
213991_s_at	-1.138	-2,20075722	Heparan sulfate (glucosamine) 3-O-sulfotransferase 1	HS3ST1	4p16	---
206104_at	-1.132	-2,19162353	ISL1 transcription factor, LIM/homeodomain, (islet-1)	ISL1	5q11.2	regulation of transcription, DNA-dependent /// development
215311_at	-1.125	-2,18101547	MRNA full length insert cDNA clone EUROIMAGE 21920	---	---	---
205085_at	-1.107	-2,15397275	origin recognition complex, subunit 1-like (yeast)	ORC1L	1p32	DNA replication /// DNA replication initiation
209590_at	-1.107	-2,15397275	Bone morphogenetic protein 7 (osteogenic protein 1)	BMP7	20q13	skeletal development /// cell differentiation /// growth
204591_at	-1.104	-2,14949835	cell adhesion molecule with homology to L1CAM (close homolog of L1)	CHL1	3p26.1	cell adhesion /// signal transduction
210364_at	-1.091	-2,13021641	sodium channel, voltage-gated, type II, beta	SCN2B	11q23	ion transport /// sodium ion transport /// synaptic transmission
211223_at	-1.09	-2,12874036	prophet of Pit1, paired-like homeodomain transcription factor	PROP1	5q35.3	regulation of transcription, DNA-dependent /// central nervous system development
214360_at	-1.079	-2,11257125	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	SMARCA4	19p13.2	transcription /// regulation of transcription from RNA polymerase II promoter
209612_s_at	-1.078	-2,11110744	alcohol dehydrogenase IB (class I), beta polypeptide	ADH1B	4q21-q23	ethanol oxidation
211831_s_at	-1.076	-2,10818285	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	THPO	3q27	development /// cell proliferation
216782_at	-1.076	-2,10818285	Potassium inwardly-rectifying channel, subfamily J, member 15	KCNJ15	21q22.2	ion transport /// potassium ion transport
203431_s_at	-1.072	-2,10234582	Rho GTPase-activating protein	RICS	11q24-q25	---
210702_s_at	-1.069	-2,09797866	prostaglandin I2 (prostacyclin) synthase	PTGIS	20q13.13	prostaglandin biosynthesis /// electron transport /// lipid metabolism /// fatty acid biosynthesis
205962_at	-1.067	-2,09507225	p21 (CDKN1A)-activated kinase 2	PAK2	3q29	protein amino acid phosphorylation /// protein amino acid phosphorylation /// negative regulation of protein kinase activity /// signal transduction
205754_at	-1.067	-2,09507225	coagulation factor II (thrombin)	F2	11p11-q12	regulation of cell cycle /// proteolysis and peptidolysis /// apoptosis /// caspase activation /// acute-phase response /// tyrosine phosphorylation of STAT protein /// STAT protein nuclear translocation /// development /// response to wounding /// platel
210359_at	-1.066	-2,09362056	metastasis suppressor 1	MTSS1	8p22	cell motility /// cell cycle /// cell adhesion /// transmembrane receptor protein tyrosine kinase signaling pathway /// neurogenesis /// muscle development /// microspike biogenesis /// actin cytoskeleton organization and biogenesis /// negative regulatio
216132_at	-1.045	-2,06336636	Astrotactin 2	ASTN2	9q33.1	---
209437_s_at	-1.038	-2,05337909	spondin 1, extracellular matrix protein	SPON1	11p15.2	cell adhesion /// development
206609_at	-1.032	-2,04485706	melanoma antigen family C, 1	MAGEC1	Xq26	---
216801_at	-1.03	-2,04202425	hypothetical gene supported by BC033316 /// hypothetical gene supported by BC033316	LOC400742 /// LOC440572	1p36.13	---
203499_at	-1.028	-2,03919537	EPH receptor A2	EPHA2	1p36	protein amino acid phosphorylation /// signal transduction /// transmembrane receptor protein tyrosine kinase signaling pathway /// development
220471_s_at	-1.025	-2,03495938	myc target 1	MYCT1	6q25.2	---
214590_s_at	-1.018	-2,02510961	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	UBE2D1	10q11.2-q21	ubiquitin-dependent protein catabolism /// ubiquitin cycle
220425_x_at	-1.018	-2,02510961	ropporin, rhophilin associated protein 1B	ROPN1B	3q21.2	cytokinesis /// signal transduction /// Rho protein signal transduction /// spermatogenesis /// acrosome reaction /// fusion of sperm to egg plasma membrane /// cell-cell adhesion /// sperm motility
216420_at	-1.015	-2,02090289	---	---	---	---
205122_at	-1.009	-2,01251565	transmembrane protein with EGF-like and two follistatin-like domains 1	TMEFF1	9q31	---

206803_at	-1.007	-2,00972764	prodynorphin	PDYN	20pter-p12	neuropeptide signaling pathway /// synaptic transmission
205072_s_at	-1.004	-2,00555287	X-ray repair complementing defective repair in Chinese hamster cells 4	XRCC4	5q13-q14	DNA repair /// double-strand break repair /// DNA recombination /// DNA recombination

Tab. A3d:
Probe-sets down-regulated regulated upon IVIG treatment at day 21 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A3e

Probe Set ID	log2 (fold change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
216740_at	2.129	4,37414183	Transcriptional regulating factor 1	TRERF1	6p21.1-p12.1	regulation of transcription, DNA-dependent /// steroid biosynthesis /// cholesterol catabolism /// development /// homeostasis /// positive regulation of transcription, DNA-dependent /// regulation of hormone biosynthesis
204531_s_at	1.947	3,85571923	breast cancer 1, early onset	BRCA1	17q21	cell cycle checkpoint /// DNA repair /// regulation of transcription from RNA polymerase II promoter /// regulation of transcription from RNA polymerase III promoter /// DNA damage response, signal transduction by p53 class mediator resulting in transcrip
204414_at	1.823	3,53816174	---	---	---	---
222258_s_at	1.816	3,52103605	SH3-domain binding protein 4	SH3BP4	2q37.1-q37.2	endocytosis /// cell cycle
215554_at	1.748	3,35892597	glycosylphosphatidylinositol specific phospholipase D1	GPLD1	6p22.3-p22.2	cell-matrix adhesion
220258_s_at	1.702	3,25351679	hypothetical protein FLJ10385	FLJ10385	17p13.1	---
214019_at	1.631	3,09727611	---	---	---	---
203702_s_at	1.558	2,94445372	tubulin tyrosine ligase-like family, member 4	TTLL4	2p24.3-p24.1	protein modification
206366_x_at	1.542	2,9119791	chemokine (C motif) ligand 2	XCL2	1q23-q25	chemotaxis /// immune response /// signal transduction /// cell-cell signaling /// sensory perception /// circulation
216258_s_at	1.534	2,89587634	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 13	SERPINB13	18q21.3-q22	response to UV /// regulation of proteolysis and peptidolysis
222202_at	1.518	2,86393748	CDNA FLJ14293 fis, clone PLACE1007866	---	---	---
210066_s_at	1.493	2,81473675	aquaporin 4	AQP4	18q11.2-q12.1	transport /// neurogenesis /// excretion
217452_s_at	1.469	2,76829943	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	B3GALT2	1q31	protein amino acid glycosylation
212396_s_at	1.467	2,76446441	KIAA0090	KIAA0090	1p36.13	---
204936_at	1.42	2,67585511	mitogen-activated protein kinase kinase kinase 2	MAP4K2	11q13	protein amino acid phosphorylation /// vesicle targeting /// response to stress /// immune response /// protein kinase cascade /// JNK cascade /// hemocyte development
222074_at	1.408	2,65369028	uroporphyrinogen decarboxylase	UROD	1p34	heme biosynthesis
219203_at	1.38	2,60268371	chromosome 14 open reading frame 122	C14orf122	14q11.2	---
215657_at	1.376	2,59547753	Solute carrier family 26, member 3	SLC26A3	7q31	transport /// anion transport /// excretion /// sulfate transport
221487_s_at	1.355	2,55797116	endosulfine alpha	ENSA	1q21.2	transport /// response to nutrients
212258_s_at	1.313	2,48457656	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	SMARCA2	9p22.3	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription from RNA polymerase II promoter /// cell cycle
204896_s_at	1.304	2,46912521	prostaglandin E receptor 4 (subtype EP4)	PTGER4	5p13.1	immune response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// G-protein signaling, coupled to cAMP nucleotide second messenger
214126_at	1.294	2,45206972	Mitochondrial carrier triple repeat 1	MCART1	9p13.3-p12	transport
217566_s_at	1.279	2,42670712	transglutaminase 4 (prostate)	TGM4	3p22-p21.33	peptide cross-linking /// protein amino acid polyamination
207228_at	1.269	2,40994463	protein kinase, cAMP-dependent, catalytic, gamma	PRKACG	9q13	protein amino acid phosphorylation /// spermatogenesis /// male gonad development
201961_s_at	1.257	2,38998241	ring finger protein 41	RNF41	12q13.2-q13.3	protein ubiquitination
208198_x_at	1.247	2,3734736	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 1	KIR2DS1	19q13.4	immune response

208906_at	1.244	2,36854322	Bernardinelli-Seip congenital lipodystrophy 2 (seipin) /// hypothetical protein DKFZp762N1910	BSCL2 /// DKFZp762N1910	11q12-q13.5 /// 11q12.3	---
211140_s_at	1.229	2,34404457	caspase 2, apoptosis-related cysteine protease (neural precursor cell expressed, developmentally down-regulated 2)	CASP2	7q34-q35	proteolysis and peptidolysis /// proteolysis and peptidolysis /// anti-apoptosis /// apoptotic program /// regulation of apoptosis
216896_at	1.159	2,23302592	collagen, type IV, alpha 3 (Goodpasture antigen)	COL4A3	2q36-q37	proteolysis and peptidolysis /// phosphate transport /// induction of apoptosis /// caspase activation /// cell adhesion /// cell surface receptor linked signal transduction /// perception of sound /// circulation /// cell proliferation /// negative regul
217082_at	1.158	2,23147864	Unknown protein	---	---	---
214160_at	1.158	2,23147864	---	---	---	---
221968_s_at	1.138	2,20075722	Mesenchymal stem cell protein DSC43	LOC51333	16p11.2	---
214579_at	1.128	2,18555548	hypothetical protein dJ462O23.2	DJ462O23.2	1p36.12-p35.1	---
202563_at	1.121	2,17497678	chromosome 14 open reading frame 1	C14orf1	14q24.3	sterol biosynthesis
220448_at	1.114	2,16444929	potassium channel, subfamily K, member 12	KCNK12	2p22-p21	ion transport /// potassium ion transport
214507_s_at	1.109	2,15696086	exosome component 2	EXOSC2	9q34	rRNA processing
220070_at	1.099	2,14206165	hypothetical protein FLJ13798	FLJ13798	16p12.1	---
203862_s_at	1.093	2,13317156	actinin, alpha 2	ACTN2	1q42-q43	---
209346_s_at	1.09	2,12874036	phosphatidylinositol 4-kinase type II	PI4KII	10q24	phosphatidylinositol biosynthesis
204056_s_at	1.07	2,09943337	mevalonate kinase (mevalonic aciduria)	MVK	12q24	protein amino acid phosphorylation /// cholesterol biosynthesis /// isoprenoid biosynthesis
218890_x_at	1.069	2,09797866	mitochondrial ribosomal protein L35	MRPL35	2p11.2	---
208546_x_at	1.056	2,07915887	histone 1, H2bh	HIST1H2BH	6p21.3	nucleosome assembly /// nucleosome assembly /// chromosome organization and biogenesis (sensu Eukaryota)
210415_s_at	1.055	2,07771821	outer dense fiber of sperm tails 2	ODF2	9q34.11	---
214530_x_at	1.048	2,06766147	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	EPB41	1p33-p32	circulation /// cortical actin cytoskeleton organization and biogenesis
215645_at	1.046	2,06479707	Hypothetical protein MGC13008	FLCN	17p11.2	---
221713_s_at	1.039	2,05480288	hypothetical protein FLJ12748 /// hypothetical protein FLJ12748	FLJ12748	3q27.1	---
208029_s_at	1.037	2,05195629	lysosomal associated protein transmembrane 4 beta /// lysosomal associated protein transmembrane 4 beta	LAPTM4B	8q22.1	---
219408_at	1.037	2,05195629	protein arginine N-methyltransferase 7	PRMT7	16q22.1	---
206863_x_at	1.028	2,03919537	---	---	---	---
215409_at	1.025	2,03495938	PLSC domain containing protein	LOC254531	15q14	metabolism
206519_x_at	1.016	2,02230416	---	---	---	---
215965_at	1.009	2,01251565	CDNA FLJ12359 fis, clone MAMMA1002355	---	---	---
215767_at	1.002	2,00277451	chromosome 2 open reading frame 10	C2orf10	2q32.1	---
214095_at	1.002	2,00277451	serine hydroxymethyltransferase 2 (mitochondrial)	SHMT2	12q12-q14	glycine metabolism /// L-serine metabolism /// one-carbon compound metabolism

Tab. A3e:
Probe-sets up-regulated regulated upon IVIG treatment at day 21 compared to day 6;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A3f

Probe Set ID	log2 (fold change)	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
215076_s_at	-1.958	-3,88522998	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	COL3A1	2q31	phosphate transport /// circulation /// organogenesis
208153_s_at	-1.845	-3,59252949	FAT tumor suppressor homolog 2 (Drosophila) /// FAT tumor suppressor homolog 2 (Drosophila)	FAT2	5q32-q33	cell adhesion /// homophilic cell adhesion
215312_at	-1.836	-3,57018789	DNA damage repair and recombination protein RAD52 pseudogene	---	---	---
219789_at	-1.703	-3,25577274	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	NPR3	5p14-p13	skeletal development
205249_at	-1.686	-3,21763348	early growth response 2 (Krox-20 homolog, Drosophila)	EGR2	10q21.1	transcription /// regulation of transcription, DNA-dependent /// brain development /// peripheral nervous system development /// mechanosensory behavior
213458_at	-1.62	-3,07375036	KIAA0974	KIAA0974	10q22.2	---
206265_s_at	-1.62	-3,07375036	glycosylphosphatidylinositol specific phospholipase D1	GPLD1	6p22.3-p22.2	cell-matrix adhesion
206407_s_at	-1.613	-3,05887256	chemokine (C-C motif) ligand 13	CCL13	17q11.2	calcium ion homeostasis /// chemotaxis /// inflammatory response /// signal transduction /// cell-cell signaling /// sensory perception
216474_x_at	-1.554	-2,93630127	tryptase alpha/beta 1 /// tryptase beta 2	TPSAB1 /// TPSB2	16p13.3	proteolysis and peptidolysis /// defense response /// proteolysis and peptidolysis
209819_at	-1.554	-2,93630127	hyaluronan binding protein 4	HABP4	9q22.3-q31	---
207852_at	-1.546	-2,92006402	chemokine (C-X-C motif) ligand 5	CXCL5	4q12-q13	chemotaxis /// inflammatory response /// signal transduction /// cell-cell signaling /// positive regulation of cell proliferation
215013_s_at	-1.541	-2,90996137	ubiquitin specific protease 34	USP34	2p15	ubiquitin-dependent protein catabolism /// ubiquitin cycle
220270_at	-1.521	-2,86989907	tudor domain containing 4	TDRD4	13q12.12	---
211627_x_at	-1.494	-2,81668845	estrogen receptor 1 /// estrogen receptor 1	ESR1	6q25.1	regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// signal transduction /// cell growth /// estrogen receptor signaling pathway /// negative regulation of mitosis
211154_at	-1.468	-2,76638126	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	THPO	3q27	development /// cell proliferation
214361_s_at	-1.441	-2,71508996	regulator of G-protein signalling 12	RGS12	4p16.3	signal transduction /// regulation of G-protein coupled receptor protein signaling pathway /// protein transport
220920_at	-1.44	-2,71320865	ATPase, Class V, type 10B	ATP10B	5q34	cation transport
221091_at	-1.414	-2,66474965	insulin-like 5	INSL5	1p31.1-p22.3	physiological process
216201_at	-1.411	-2,65921422	CDNA: FLJ21586 fis, clone COL06920	---	---	---
219903_s_at	-1.407	-2,65185152	cytochrome P450, family 2, subfamily C, polypeptide 8	CYP2C8	10q23.33	electron transport /// electron transport /// transport
216206_x_at	-1.406	-2,65001403	mitogen-activated protein kinase kinase 7	MAP2K7	19p13.3-p13.2	protein amino acid phosphorylation /// response to stress /// signal transduction
206446_s_at	-1.401	-2,64084568	elastase 2A	ELA2A	1p36.21	proteolysis and peptidolysis
206414_s_at	-1.396	-2,63170905	development and differentiation enhancing factor 2	DDEF2	2p25|2p24	regulation of GTPase activity
207836_s_at	-1.376	-2,59547753	RNA binding protein with multiple splicing	RBPMS	8p12-p11	RNA processing
208222_at	-1.342	-2,53502504	activin A receptor, type IB	ACVR1B	12q13	protein amino acid phosphorylation /// transmembrane receptor protein serine/threonine kinase signaling pathway
204694_at	-1.34	-2,53151319	alpha-fetoprotein	AFP	4q11-q13	transport /// immune response
214451_at	-1.334	-2,52100678	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	TFAP2B	6p21-p12	transcription /// regulation of transcription from RNA polymerase II promoter /// neurogenesis
216329_at	-1.331	-2,51576994	---	---	---	---
201042_at	-1.327	-2,50880441	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	TGM2	20q12	G-protein coupled receptor protein signaling pathway /// peptide cross-linking /// positive regulation of cell adhesion
209866_s_at	-1.325	-2,50532888	latrophilin 3	LPHN3	4q13.1	signal transduction /// neuropeptide signaling pathway
215850_s_at	-1.318	-2,49320239	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	NDUFA5	7q32	electron transport
216815_at	-1.316	-2,48974847	---	---	---	---
206294_at	-1.307	-2,47426496	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	HSD3B2	1p13.1	C21-steroid hormone biosynthesis
220382_s_at	-1.286	-2,43851019	Rho GTPase activating protein 28	ARHGAP28	18p11.23	viral release
222246_at	-1.262	-2,39827983	Syntaxin binding protein 3	STXBP3	1p13.3	vesicle docking during exocytosis /// protein transport /// vesicle-mediated transport
216420_at	-1.255	-2,38667149	---	---	---	---

211224_s_at	-1.226	-2,33917533	ATP-binding cassette, sub-family B (MDR/TAP), member 11	ABCB11	2q24	transport
205498_at	-1.207	-2,30857084	growth hormone receptor	GHR	5p13-p12	skeletal development /// endocytosis /// growth
206903_at	-1.205	-2,30537269	---	---	---	---
206043_s_at	-1.202	-2,30058379	KIAA0703 gene product	KIAA0703	16q24.1	cation transport /// calcium ion transport /// metabolism /// proton transport
214708_at	-1.191	-2,28310941	syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)	SNTB1	8q23-q24	muscle contraction
214689_at	-1.189	-2,27994655	pappalysin 2	PAPPA2	1q23-q25	regulation of cell growth /// regulation of cell growth /// proteolysis and peptidolysis /// proteolysis and peptidolysis /// cell differentiation
222383_s_at	-1.18	-2,26576777	arachidonate lipoxygenase 3	ALOXE3	17p13.1	electron transport /// leukotriene biosynthesis
220223_at	-1.157	-2,22993244	hypothetical protein FLJ12735	FLJ12735	17q11.2	protein folding
207567_at	-1.153	-2,22375832	solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2	SLC13A2	17p13.2	ion transport /// sodium ion transport
215911_x_at	-1.148	-2,2160647	ATPase, Ca++ transporting, plasma membrane 3	ATP2B3	Xq28	cation transport /// calcium ion transport /// metabolism
220687_at	-1.144	-2,20992897	---	---	---	---
214716_at	-1.141	-2,20533833	BMP2 inducible kinase	BMP2K	4q21.21	protein amino acid phosphorylation
213862_at	-1.136	-2,19770844	Patatin-like phospholipase domain containing 2	PNPLA2	11p15.5	---
204406_at	-1.111	-2,15995312	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT1	13q12	angiogenesis /// protein amino acid phosphorylation /// transmembrane receptor protein tyrosine kinase signaling pathway /// pregnancy /// positive regulation of cell proliferation /// cell differentiation
209676_at	-1.109	-2,15696086	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	TFPI	2q31-q32.1	blood coagulation
207834_at	-1.102	-2,14652057	fibulin 1	FBLN1	22q13.31	chitin metabolism /// development
207333_at	-1.101	-2,14503323	neuromedin B receptor	NMBR	6q21-qter	signal transduction /// G-protein signaling, coupled to IP3 second messenger (phospholipase C activating)
206951_at	-1.098	-2,1405774	histone 1, H4e	HIST1H4E	6p21.3	---
216280_s_at	-1.086	-2,12284642	Dicer1, Dcr-1 homolog (Drosophila)	DICER1	14q32.13	RNA processing /// RNA interference, targeting of mRNA for destruction
219534_x_at	-1.085	-2,12137548	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
206609_at	-1.084	-2,11990557	melanoma antigen family C, 1	MAGEC1	Xq26	---
219984_s_at	-1.059	-2,08348686	HRAS-like suppressor	HRASLS	3q29	---
211334_at	-1.057	-2,08060053	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	MRE11A	11q21	regulation of mitotic recombination /// double-strand break repair via nonhomologous end-joining /// telomerase-dependent telomere maintenance /// meiosis /// meiotic recombination
204677_at	-1.053	-2,07483987	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
215844_at	-1.049	-2,06909516	transportin 2 (importin 3, karyopherin beta 2b)	TNPO2	19p13.13	protein-nucleus import, docking /// protein transport
215435_at	-1.035	-2,04911365	Development and differentiation enhancing factor 1	DDEF1	8q24.1-q24.2	regulation of GTPase activity
210810_s_at	-1.03	-2,04202425	solute carrier family 6 (neurotransmitter transporter, glycine), member 5	SLC6A5	11p15.2-p15.1	neurotransmitter transport /// synaptic transmission
201616_s_at	-1.027	-2,03778239	caldesmon 1	CALD1	7q33	muscle contraction /// muscle development
207201_s_at	-1.026	-2,0363704	solute carrier family 22 (organic cation transporter), member 1	SLC22A1	6q26	ion transport /// sodium ion transport /// organic cation transport
201124_at	-1.014	-2,0195026	integrin, beta 5	ITGB5	3q21.2	cell-matrix adhesion /// integrin-mediated signaling pathway /// development
222269_at	-1.011	-2,01530752	chromosome X open reading frame 33	CXorf33	Xq21.1	---
210795_s_at	-1.007	-2,00972764	Maternally expressed 3	MEG3	14q32	---

Tab. A3f:
Probe-sets down-regulated regulated upon IVIG treatment at day 21 compared to day 6;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. 4a

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosoma Location	GO Biological Process Description
210313_at	-3.526	-11,52	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 4	ILT7	19q13.4	immune response
215047_at	-3.282	-9,73	tripartite motif-containing 58	TRIM58	1q44	protein ubiquitination
207989_at	-2.775	-6,84	---	---	---	---
212187_x_at	-2.77	-6,82	prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep
211748_x_at	-2.466	-5,53	prostaglandin D2 synthase 21kDa (brain) /// prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep
207781_s_at	-2.368	-5,16	zinc finger protein 6 (CMPX1)	ZNF6	Xq13-q21.1	regulation of transcription, DNA-dependent /// regulation of transcription
211812_s_at	-2.336	-5,05	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 3	B3GALT 3	3q25	protein amino acid glycosylation
220803_at	-2.3	-4,92	Associated molecule with the SH3 domain of STAM (AMSH) like protein	AMSH-LP	10q23.31	---
218801_at	-2.28	-4,88	UDP-glucose ceramide glucosyltransferase-like 2	UGCGL2	13q32.1	protein amino acid glycosylation /// posttranslational protein folding
210928_at	-2.258	-4,78	CCR4-NOT transcription complex, subunit 2	CNOT2	12q15	regulation of transcription, DNA-dependent /// regulation of global transcription from RNA polymerase II promoter
222258_s_at	-2.248	-4,75	SH3-domain binding protein 4	SH3BP4	2q37.1-q37.2	endocytosis /// cell cycle
204444_at	-2.206	-4,61	kinesin family member 11	KIF11	10q24.1	cytokinesis /// microtubule-based movement /// cell cycle /// mitotic spindle organization and biogenesis
204525_at	-2.162	-4,48	PHD finger protein 14	PHF14	7p21.3	regulation of transcription, DNA-dependent
215613_at	-2.135	-4,39	A disintegrin and metalloproteinase domain 12 (meltrin alpha)	ADAM12	10q26.3	proteolysis and peptidolysis /// cell adhesion /// myoblast fusion
219387_at	-2.132	-4,38	KIAA1212	KIAA121 2	2p16.3	---
207744_at	-2.129	-4,37	---	---	---	---
211520_s_at	-2.064	-4,18	glutamate receptor, ionotropic, AMPA 1	GRIA1	5q33|5q31.1	ion transport /// potassium ion transport /// signal transduction /// synaptic transmission
213568_at	-2.055	-4,16	odd-skipped related 2 (Drosophila)	OSR2	8q22.2	---
203753_at	-2.036	-4,10	transcription factor 4	TCF4	18q21.1	transcription /// regulation of transcription from RNA polymerase II promoter
215695_s_at	-2.027	-4,08	glycogenin 2	GYG2	Xp22.3	glycogen biosynthesis /// carbohydrate biosynthesis
211174_s_at	-2.024	-4,07	cholecystokinin A receptor	CCKAR	4p15.1-p15.2	smooth muscle contraction /// signal transduction /// positive regulation of cytosolic calcium ion concentration /// response to nutrients /// digestion /// feeding behavior
207539_s_at	-2.008	-4,02	interleukin 4	IL4	5q31.1	chemotaxis /// cellular defense response /// cholesterol metabolism /// cell proliferation /// B-cell differentiation /// T helper 2 type immune response /// connective tissue growth factor biosynthesis /// regulation of isotype switching
213395_at	-1.994	-3,98	megalencephalic leukoencephalopathy with subcortical cysts 1	MLC1	22q13.33	protein biosynthesis /// ion transport
206637_at	-1.993	-3,98	purinergic receptor P2Y, G-protein coupled, 14	P2RY14	3q21-q25	signal transduction /// G-protein coupled receptor protein signaling pathway
204939_s_at	-1.993	-3,98	phospholamban	PLN	6q22.1	calcium ion transport /// muscle contraction /// circulation
209981_at	-1.973	-3,93	RNA-binding protein pippin	PIPPIN	22q13.2-q13.31	regulation of transcription, DNA-dependent /// mRNA processing /// histone mRNA 3'-end processing
216894_x_at	-1.971	-3,92	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
219534_x_at	-1.966	-3,91	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
221062_at	-1.963	-3,90	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	HS3ST3 B1	17p12-p11.2	heparan sulfate proteoglycan biosynthesis, enzymatic modification
209655_s_at	-1.96	-3,89	transmembrane protein 47	TMEM47	Xp11.4	---
214660_at	-1.957	-3,88	Integrin, alpha 1	PELO	5q11.2	protein biosynthesis
216623_x_at	-1.916	-3,77	trinucleotide repeat containing 9	TNRC9	16q12.1	regulation of transcription, DNA-dependent
216613_at	-1.914	-3,77	MRNA; cDNA DKFZp566L0824 (from clone DKFZp566L0824)	---	---	---
215767_at	-1.911	-3,76	chromosome 2 open reading frame 10	C2orf10	2q32.1	---
203443_at	-1.904	-3,74	hypothetical protein FLJ35827	FLJ3582 7	11q12.3	---
205378_s_at	-1.89	-3,71	acetylcholinesterase (YT blood group)	ACHE	7q22	acetylcholine catabolism in synaptic cleft /// DNA replication /// cell adhesion /// synaptogenesis /// muscle development /// cell proliferation /// response to wounding /// neurotransmitter catabolism /// amyloid precursor protein metabolism /// positiv
210572_at	-1.856	-3,62	protocadherin alpha 2	PCDHA2	5q31	cell adhesion /// homophilic cell adhesion /// neurogenesis
209989_at	-1.852	-3,61	zinc finger protein 268	ZNF268	12q24.33	transcription /// regulation of transcription, DNA-dependent

200878_at	-1.844	-3,59	endothelial PAS domain protein 1	EPAS1	2p21-p16	angiogenesis /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// signal transduction /// cell differentiation
213426_s_at	-1.799	-3,48	caveolin 2	CAV2	7q31.1	---
216468_s_at	-1.781	-3,44	zinc finger protein 682	ZNF682	19p12	regulation of transcription, DNA-dependent
205990_s_at	-1.778	-3,43	wingless-type MMTV integration site family, member 5A	WNT5A	3p21-p14	signal transduction /// frizzled-2 signaling pathway /// cell-cell signaling /// morphogenesis
217757_at	-1.762	-3,39	alpha-2-macroglobulin	A2M	12p13.3-p12.3	intracellular protein transport /// protein homooligomerization
219661_at	-1.761	-3,39	RAN binding protein 17	RANBP17	5q34	protein-nucleus import, docking /// protein transport
206065_s_at	-1.75	-3,36	dihydropyrimidinase	DPYS	8q22	nucleobase, nucleoside, nucleotide and nucleic acid metabolism /// response to toxin
221086_s_at	-1.741	-3,34	zinc finger protein 312	ZNF312	3p14.2	---
204753_s_at	-1.732	-3,32	hepatic leukemia factor	HLF	17q22	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// development /// rhythmic process
213966_at	-1.715	-3,28	High-mobility group 20B	HMG20B	19p13.3	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// cell cycle /// chromatin modification
213182_x_at	-1.696	-3,24	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
205533_s_at	-1.669	-3,18	cadherin 6, type 2, K-cadherin (fetal kidney)	CDH6	5p15.1-p14	cell adhesion /// homophilic cell adhesion
216180_s_at	-1.667	-3,18	synaptojanin 2	SYNJ2	6q25.3	---
220774_at	-1.661	-3,16	dymeclin	DYM	18q12-q21.1	---
221397_at	-1.659	-3,16	taste receptor, type 2, member 10	TAS2R10	12p13	signal transduction /// G-protein coupled receptor protein signaling pathway /// sensory perception /// perception of taste
210726_at	-1.647	-3,13	cytochrome P450, family 3, subfamily A, polypeptide 4	CYP3A4	7q21.1	electron transport /// lipid metabolism /// xenobiotic metabolism /// xenobiotic metabolism /// transport
214702_at	-1.632	-3,10	fibronectin 1	FN1	2q34	acute-phase response /// cell adhesion /// metabolism /// response to wounding /// cell migration
220061_at	-1.615	-3,06	hypothetical protein FLJ20581	FLJ20581	16p12.3	metabolism
207156_at	-1.614	-3,06	histone 1, H2ag	HIST1H2AG	6p22.1	---
216291_at	-1.606	-3,04	---	---	---	---
220911_s_at	-1.599	-3,03	KIAA1305	KIAA1305	14q11.2	DNA recombination
210228_at	-1.579	-2,99	colony stimulating factor 2 (granulocyte-macrophage)	CSF2	5q31.1	cellular defense response /// cell surface receptor linked signal transduction /// development
216644_at	-1.578	-2,99	CDNA FLJ20178 fis, clone COL09990	---	---	---
207481_at	-1.571	-2,97	---	---	---	---
210397_at	-1.57	-2,97	defensin, beta 1	DEFB1	8p23.2-p23.1	response to pest, pathogen or parasite /// defense response to bacteria /// innate immune response /// innate immune response
216681_at	-1.561	-2,95	---	---	---	---
222172_at	-1.559	-2,95	neuronal PAS domain protein 3	NPAS3	14q12-q13	transcription /// regulation of transcription, DNA-dependent /// signal transduction
201911_s_at	-1.557	-2,94	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	FARP1	13q32.2	---
215105_at	-1.554	-2,94	hypothetical gene CG030	CG030	13q12-q13	---
209544_at	-1.545	-2,92	receptor-interacting serine-threonine kinase 2	RIPK2	8q21	protein amino acid phosphorylation /// inflammatory response /// signal transduction /// regulation of apoptosis /// positive regulation of I-kappaB kinase/NF-kappaB cascade
218856_at	-1.54	-2,91	tumor necrosis factor receptor superfamily, member 21	TNFRSF21	6p21.1-12.2	apoptosis /// signal transduction
217400_at	-1.524	-2,88	---	---	---	---
201820_at	-1.52	-2,87	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)	KRT5	12q12-q13	epidermis development
211412_at	-1.518	-2,86	peptidyl arginine deiminase, type IV	PADI4	1p36.13	protein modification /// protein modification
222025_s_at	-1.518	-2,86	5-oxoprolinase (ATP-hydrolysing)	OPLAH	8q24.3	---
206796_at	-1.504	-2,84	WNT1 inducible signaling pathway protein 1	WISP1	8q24.1-q24.3	regulation of cell growth /// cell adhesion /// signal transduction /// cell-cell signaling /// Wnt receptor signaling pathway
201906_s_at	-1.486	-2,80	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	CTDSPL	3p21.3	---
210164_at	-1.485	-2,80	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) /// granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	14q11.2	proteolysis and peptidolysis /// apoptosis /// cleavage of lamin /// cytolysis
211381_x_at	-1.462	-2,75	sperm associated antigen 11	SPAG11	8p23-p22	defense response /// spermatogenesis /// response to pest, pathogen or parasite
201741_x_at	-1.46	-2,75	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	SFRS1	17q21.3-q22	nuclear mRNA splicing, via spliceosome /// mRNA splice site selection
222298_at	-1.452	-2,74	Coatomer protein complex, subunit gamma 2	COPG2	7q32	retrograde transport, Golgi to ER /// intra Golgi transport /// protein transport
211663_x_at	-1.433	-2,70	prostaglandin D2 synthase 21kDa (brain) /// prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep

209135_at	-1.433	-2,70	aspartate beta-hydroxylase	ASPH	8q12.1	muscle contraction /// peptidyl-amino acid modification
206661_at	-1.43	-2,69	Dbf4-related factor 1	DRF1	17q21.31	---
210956_at	-1.428	-2,69	pancreatic polypeptide receptor 1	PPYR1	10q11.2	signal transduction /// G-protein coupled receptor protein signaling pathway /// digestion /// feeding behavior /// circulation
219851_at	-1.427	-2,69	zinc finger protein 613	ZNF613	19q13.41	regulation of transcription, DNA-dependent
210545_at	-1.419	-2,67	intersectin 2	ITSN2	2pter-p25.1	endocytosis
206742_at	-1.384	-2,61	c-fos induced growth factor (vascular endothelial growth factor D)	FIGF	Xp22.31	regulation of cell cycle /// angiogenesis /// cell proliferation /// positive regulation of cell proliferation /// cell differentiation
210261_at	-1.381	-2,60	potassium channel, subfamily K, member 2	KCNK2	1q41	ion transport /// potassium ion transport /// potassium ion transport
204817_at	-1.375	-2,59	extra spindle poles like 1 (S. cerevisiae)	ESPL1	12q	mitotic sister chromatid segregation /// regulation of cell cycle /// cytokinesis /// proteolysis and peptidolysis /// apoptosis /// chromosome segregation /// establishment of mitotic spindle localization /// positive regulation of mitotic metaphase/anap
220903_at	-1.361	-2,57	G elongation factor, mitochondrial 1	GFM1	3q25.1-q26.2	protein biosynthesis /// translational elongation
220790_s_at	-1.352	-2,55	membrane-spanning 4-domains, subfamily A, member 5	MS4A5	11q12	signal transduction
213462_at	-1.33	-2,51	neuronal PAS domain protein 2	NPAS2	2q11.2	transcription /// regulation of transcription, DNA-dependent /// signal transduction /// central nervous system development
215655_at	-1.328	-2,51	Glutamate receptor, ionotropic, kainate 2	GRIK2	6q16.3-q21	ion transport /// potassium ion transport /// glutamate signaling pathway /// synaptic transmission /// synaptic transmission
206619_at	-1.305	-2,47	dickkopf homolog 4 (Xenopus laevis)	DKK4	8p11.2-p11.1	development /// Wnt receptor signaling pathway /// negative regulation of Wnt receptor signaling pathway
206024_at	-1.298	-2,46	4-hydroxyphenylpyruvate dioxygenase	HPD	12q24-qter	L-phenylalanine catabolism /// tyrosine catabolism /// aromatic amino acid family metabolism
220200_s_at	-1.293	-2,45	PR/SET domain containing protein 8	SET8	12q24.31	chromatin modification
211813_x_at	-1.286	-2,44	decorin	DCN	12q21.33	organogenesis
219463_at	-1.283	-2,43	chromosome 20 open reading frame 103	C20orf103	20p12	---
215406_at	-1.282	-2,43	Triple functional domain (PTPRF interacting)	TRIO	5p15.1-p14	protein amino acid phosphorylation /// transmembrane receptor protein tyrosine phosphatase signaling pathway
216023_at	-1.279	-2,43	jumonji domain containing 2B	JMJD2B	19p13.3	regulation of transcription, DNA-dependent
213997_at	-1.279	-2,43	KIAA0574 protein	KIAA0574	15q12	---
217276_x_at	-1.274	-2,42	serine hydrolase-like 2	SERHL2	22q13	aromatic compound metabolism
202837_at	-1.26	-2,39	FLN29 gene product	FLN29	12q	---
207399_at	-1.259	-2,39	beaded filament structural protein 2, phakinin	BFSP2	3q21-q25	sensory perception /// visual perception
216991_at	-1.237	-2,36	zinc finger protein 224	ZNF224	19q13.2	transcription /// regulation of transcription, DNA-dependent
211670_x_at	-1.228	-2,34	synovial sarcoma, X breakpoint 3 /// synovial sarcoma, X breakpoint 3	SSX3	Xp11.23	transcription /// regulation of transcription, DNA-dependent
217511_at	-1.217	-2,32	Kazal-type serine protease inhibitor domain 1	KAZALD1	10q24.32	regulation of cell growth
203214_x_at	-1.206	-2,31	cell division cycle 2, G1 to S and G2 to M	CDC2	10q21.1	cytokinesis /// protein amino acid phosphorylation /// mitosis /// traversing start control point of mitotic cell cycle
221377_s_at	-1.193	-2,29	recombining binding protein suppressor of hairless (Drosophila)-like	RBPSUHL	20q12-q13.1	transcription /// regulation of transcription, DNA-dependent /// signal transduction
202684_s_at	-1.187	-2,28	RNA (guanine-7-) methyltransferase	RNMT	18p11.22-p11.23	mRNA capping
203220_s_at	-1.181	-2,27	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	9q21.32	regulation of transcription, DNA-dependent /// signal transduction /// frizzled signaling pathway /// development /// organogenesis
206578_at	-1.171	-2,25	NK2 transcription factor related, locus 5 (Drosophila)	NKX2-5	5q34	negative regulation of transcription from RNA polymerase II promoter /// development /// heart development
204765_at	-1.168	-2,25	Rho guanine nucleotide exchange factor (GEF) 5	ARHGEF5	7q33-q35	---
219937_at	-1.166	-2,24	thyrotropin-releasing hormone degrading ectoenzyme	TRHDE	12q15-q21	proteolysis and peptidolysis /// sterol depletion response, sterol regulatory element binding-protein cleavage /// signal transduction /// cell-cell signaling
209969_s_at	-1.16	-2,23	signal transducer and activator of transcription 1, 91kDa	STAT1	2q32.2	regulation of cell cycle /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// caspase activation /// intracellular signaling cascade /// I-kappaB kinase/NF-kappaB cascade /// tyrosine phosp
222274_at	-1.158	-2,23	hypothetical protein FLJ31568	FLJ31568	22q11.23	---
214418_at	-1.145	-2,21	hypothetical protein LOC196993	LOC196993	15q22.32	---
209819_at	-1.137	-2,20	hyaluronan binding protein 4	HABP4	9q22.3-q31	---
219127_at	-1.132	-2,19	hypothetical protein MGC11242	MGC11242	17q21.32	---
216079_at	-1.128	-2,19	epilepsy, progressive myoclonus type 2A, Lafora disease (laforin)	EPM2A	6q24	carbohydrate metabolism /// glycogen metabolism /// regulation of translation /// protein amino acid dephosphorylation
206981_at	-1.123	-2,18	sodium channel, voltage-gated, type IV, alpha	SCN4A	17q23-q25.3	cation transport /// sodium ion transport /// muscle contraction
216028_at	-1.115	-2,17	DKFZP564C152 protein	DKFZP564C152	11	---
216655_s_at	-1.114	-2,16	---	---	---	---
206191_at	-1.108	-2,16	ectonucleoside triphosphate diphosphohydrolase 3	ENTPD3	3p21.3	---
205626_s_at	-1.096	-2,14	calbindin 1, 28kDa	CALB1	8q21.3-q22.1	---

205898_at	-1.09	-2,13	chemokine (C-X3-C motif) receptor 1	CX3CR1	3p21[3p21.3	chemotaxis /// cellular defense response /// cell adhesion /// signal transduction /// G-protein coupled receptor protein signaling pathway
207285_x_at	-1.07	-2,10	chorionic somatomammotropin hormone-like 1	CSHL1	17q24.2	---
207658_s_at	-1.065	-2,09	forkhead box G1B /// forkhead box G1A	FOXG1B /// FOXG1A	14q12-q13 /// 14q13	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// development /// brain development /// brain development
219487_at	-1.065	-2,09	hypothetical protein FLJ23560	FLJ2356 0	12q21.2	protein folding
204677_at	-1.031	-2,04	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
204467_s_at	-1.023	-2,03	synuclein, alpha (non A4 component of amyloid precursor) /// synuclein, alpha (non A4 component of amyloid precursor)	SNCA	4q21	anti-apoptosis /// central nervous system development
204057_at	-1.018	-2,03	interferon regulatory factor 8 /// interferon regulatory factor 8	IRF8	16q24.1	negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// immune response
215409_at	-1.012	-2,02	PLSC domain containing protein	LOC254 531	15q14	metabolism
211914_x_at	-1.012	-2,02	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease) /// neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	NF1	17q11.2	cell cycle /// Ras protein signal transduction /// negative regulation of cell proliferation /// negative regulation of cell cycle
207510_at	-1.011	-2,02	bradykinin receptor B1	BDKRB1	14q32.1-q32.2	inflammatory response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// positive regulation of cytosolic calcium ion concentration
214981_at	-1.004	-2,01	Periostin, osteoblast specific factor	POSTN	13q13.3	skeletal development /// cell adhesion /// cell adhesion

Tab. A4a:
Probe-sets down-regulated regulated upon IVMP treatment at day 6 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. 4b

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
205033_s_at	2.873	7,32586949	defensin, alpha 1, myeloid-related sequence /// defensin, alpha 3, neutrophil-specific	DEFA1 /// DEFA3	8p23.1 /// 8pter-p23.3	xenobiotic metabolism /// response to pest, pathogen or parasite /// defense response to bacteria /// defense response to fungi
207846_at	2.697	6,48452096	POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	POU1F1	3p11	regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// negative regulation of cell proliferation /// organogenesis
204726_at	2.586	6,00431639	cadherin 13, H-cadherin (heart)	CDH13	16q24.2-q24.3	cell adhesion /// homophilic cell adhesion
203324_s_at	2.484	5,59446433	caveolin 2	CAV2	7q31.1	---
216427_at	2.422	5,35913441	CDNA: FLJ22786 fis, clone KAIA2150	---	---	---
220360_at	2.366	5,15509852	THAP domain containing 9	THAP9	4q21.3	---
203862_s_at	2.359	5,13014841	actinin, alpha 2	ACTN2	1q42-q43	---
215944_at	2.338	5,05601239	---	---	---	---
219872_at	2.281	4,86014717	hypothetical protein DKFZp434L142	DKFZp434L142	4q32.1	---
217196_s_at	2.276	4,84333234	KIAA1078 protein	KIAA1078	1q32.1	---
217589_at	2.249	4,75353242	RAB40A, member RAS oncogene family	RAB40A	Xq22.1	intracellular protein transport /// small GTPase mediated signal transduction /// protein transport
208329_at	2.241	4,72724619	prostate and breast cancer overexpressed 1	PBOV1	6q23-q24	---
213591_at	2.227	4,68159457	aldehyde dehydrogenase 7 family, member A1	ALDH7A1	5q31	aldehyde metabolism /// perception of sound /// metabolism
209298_s_at	2.212	4,63317122	intersectin 1 (SH3 domain protein)	ITSN1	21q22.1-q22.2	synaptic vesicle endocytosis
204286_s_at	2.209	4,62354683	phorbol-12-myristate-13-acetate-induced protein 1	PMAIP1	18q21.32	---
220090_at	2.204	4,60755057	chromosome 1 open reading frame 10	C1orf10	1q21	response to unfolded protein /// metabolism /// response to heat /// cell-cell adhesion /// cell-cell adhesion
206295_at	2.159	4,46805185	interleukin 18 (interferon-gamma-inducing factor)	IL18	11q22.2-q22.3	angiogenesis /// immune response /// cell-cell signaling /// induction of apoptosis via death domain receptors /// regulation of cell adhesion /// sleep /// chemokine biosynthesis /// T helper 2 type immune response /// interleukin-2 biosynthesis /// inte
205385_at	2.065	4,18433976	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	MDM2	12q14.3-q15	regulation of cell cycle /// negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// protein complex assembly /// negative regulation of cell proliferation /// prote
215107_s_at	2.027	4,07556479	hypothetical protein FLJ20619	FLJ20619	1p32.3	---
216814_at	1.914	3,7685251	---	---	---	---
220504_at	1.9	3,73213197	keratocan	KERA	12q22	eye morphogenesis (sensu Mammalia) /// sensory perception /// visual perception
207437_at	1.898	3,72696172	neuro-oncological ventral antigen 1	NOVA1	14q	synaptic transmission /// synaptic transmission /// locomotory behavior /// locomotory behavior /// RNA splicing /// RNA splicing
221674_s_at	1.861	3,63259367	chordin	CHRD	3q27	skeletal development /// development
206480_at	1.855	3,61751751	leukotriene C4 synthase	LTC4S	5q35	leukotriene biosynthesis
209101_at	1.848	3,60000772	connective tissue growth factor	CTGF	6q23.1	regulation of cell growth /// DNA metabolism /// cell motility /// cell adhesion /// epidermis development /// response to wounding
207256_at	1.763	3,39403161	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	MBL2	10q11.2-q21	complement activation, lectin pathway /// phosphate transport /// immune response /// complement activation, classical pathway /// response to oxidative stress
215073_s_at	1.752	3,36825181	nuclear receptor subfamily 2, group F, member 2	NR2F2	15q26	transcription /// regulation of transcription from RNA polymerase II promoter /// lipid metabolism /// signal transduction
209373_at	1.73	3,31727818	BENE protein	BENE	2q13	---
211786_at	1.715	3,28296844	tumor necrosis factor receptor superfamily, member 9 /// tumor necrosis factor receptor superfamily, member 9	TNFRSF9	1p36	induction of apoptosis /// immune response /// negative regulation of cell proliferation
220909_at	1.705	3,26028933	tripartite motif-containing 46	TRIM46	1q22	protein ubiquitination
205973_at	1.704	3,25803025	fasciculation and elongation protein zeta 1 (zygin I)	FEZ1	11q24.2	cell adhesion /// neurogenesis /// axon guidance
221308_at	1.699	3,24675832	fibroblast growth factor receptor substrate 2	FRS2	12q15	signal transduction /// transmembrane receptor protein tyrosine phosphatase signaling pathway /// G-protein coupled receptor protein signaling pathway /// fibroblast growth factor receptor signaling pathway
206537_at	1.68	3,20427951	---	---	---	---
213717_at	1.661	3,16235647	LIM domain binding 3	LDB3	10q22.3-q23.2	---
210610_at	1.615	3,06311599	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	CEACAM1	19q13.2	immune response /// pregnancy
207142_at	1.604	3,03984971	potassium inwardly-rectifying channel, subfamily J, member 3	KCNJ3	2q24.1	ion transport /// potassium ion transport
211584_s_at	1.602	3,03563851	nuclear protein, ataxia-telangiectasia locus	NPAT	11q22-q23	---
219945_at	1.596	3,02303986	DEAD (Asp-Glu-Ala-Asp) box polypeptide 25	DDX25	11q24	---
213991_s_at	1.587	3,00423985	Heparan sulfate (glucosamine) 3-O-sulfotransferase 1	HS3ST1	4p16	---
209353_s_at	1.584	2,9979992	hypothetical protein MGC16664	MGC16664	1q25.2	---

211347_at	1.578	2,98555677	CDC14 cell division cycle 14 homolog B (S. cerevisiae)	CDC14B	9q22.33	protein amino acid dephosphorylation
220843_s_at	1.534	2,89587634	gene model 83	Gm83	8q22.3	---
206898_at	1.533	2,89386877	cadherin 19, type 2	CDH19	18q22-q23	homophilic cell adhesion
216214_at	1.509	2,83824671	Clone 24504 mRNA sequence	---	---	---
213550_s_at	1.501	2,83038832	---	---	---	---
216461_at	1.5	2,82842712	CDNA FLJ20827 fis, clone ADKA03543	---	---	---
210674_s_at	1.465	2,76063471	protocadherin alpha 9 /// protocadherin alpha subfamily C, 2 /// protocadherin alpha subfamily C, 1 /// protocadherin alpha 13 /// protocadherin alpha 12 /// protocadherin alpha 11 /// protocadherin alpha 10 /// protocadherin alpha 8 /// protocadherin alp	PCDHAC2 /// PCDHAC1 /// PCDHA13 /// PCDHA12 /// PCDHA11 /// PCDHA10 /// PCDHA8 /// PCDHA7 /// PCDHA6 /// PCDHA5 /// PCDHA4 /// PCDHA3 /// PCDHA2 /// PCDHA1	5q31	cell adhesion /// homophilic cell adhesion /// cell adhesion /// neurogenesis
40489_at	1.462	2,75490009	atrophin 1	ATN1	12p13.31	central nervous system development
213381_at	1.462	2,75490009	Chromosome 10 open reading frame 72	C10orf72	10q11.23	---
214375_at	1.444	2,7207417	PTPRF interacting protein, binding protein 1 (liprin beta 1) /// similar to PTPRF interacting protein binding protein 1 isoform 1; liprin-beta 1; liprin related protein; protein-tyrosine phosphatase receptor-type f polypeptide-interacting protein- binding	PPFIBP1 /// LOC440091	12p11.23-p11.22 /// 12p11.23	cell adhesion
200770_s_at	1.433	2,70007597	laminin, gamma 1 (formerly LAMB2)	LAMC1	1q31	protein complex assembly /// cell adhesion /// endoderm development
220815_at	1.43	2,69446715	catenin (cadherin-associated protein), alpha 3	CTNNA3	10q22.2	cell adhesion
205535_s_at	1.403	2,64450921	BH-protocadherin (brain-heart)	PCDH7	4p15	cell adhesion /// homophilic cell adhesion
222363_at	1.402	2,64267681	Transcribed locus	---	---	---
215324_at	1.396	2,63170905	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	SEMA3D	7q21.11	neurogenesis /// cell differentiation
214668_at	1.383	2,60810147	chromosome 13 open reading frame 1	C13orf1	13q14	---
204696_s_at	1.381	2,60448838	cell division cycle 25A	CDC25A	3p21	regulation of cyclin dependent protein kinase activity /// cytokinesis /// protein amino acid dephosphorylation /// mitosis /// cell proliferation
214905_at	1.363	2,57219497	hypothetical protein LOC145899	LOC145899	15q24.2	---
210852_s_at	1.36	2,5668518	aminoadipate-semialdehyde synthase	AASS	7q31.3	electron transport /// lysine catabolism /// protein tetramerization
219537_x_at	1.357	2,56151972	delta-like 3 (Drosophila)	DLL3	19q13	skeletal development /// embryonic development (sensu Mammalia) /// cell fate determination /// Notch signaling pathway /// Notch signaling pathway /// development /// neurogenesis /// cell differentiation
211531_x_at	1.335	2,52275482	proline-rich protein BstNI subfamily 1 /// proline-rich protein BstNI subfamily 2	PRB1 /// PRB2	12p13.2	G-protein coupled receptor protein signaling pathway
81737_at	1.318	2,49320239	Similar to Group X secretory phospholipase A2 precursor (Phosphatidylcholine 2-acylhydrolase GX) (GX sPLA2) (sPLA2-X)	---	16p13.11	---
220425_x_at	1.315	2,48802331	ropporin, rhophilin associated protein 1B	ROPN1B	3q21.2	cytokinesis /// signal transduction /// Rho protein signal transduction /// spermatogenesis /// acrosome reaction /// fusion of sperm to egg plasma membrane /// cell-cell adhesion /// sperm motility
204541_at	1.31	2,4794154	SEC14-like 2 (S. cerevisiae)	SEC14L2	22q12.2	transcription /// transport /// intracellular protein transport /// regulation of cholesterol biosynthesis /// positive regulation of transcription, DNA-dependent
217565_at	1.308	2,47598058	glutamate receptor, ionotrophic, AMPA 3	GRIA3	Xq25-q26	ion transport /// potassium ion transport /// glutamate signaling pathway /// synaptic transmission
219744_at	1.299	2,46058289	fructosamine 3 kinase	FN3K	17q25.3	fructoselysine metabolism
212942_s_at	1.253	2,38336515	KIAA1199	KIAA1199	15q24	perception of sound
220277_at	1.243	2,36690204	CXXC finger 4	CXXC4	4q22-q24	negative regulation of Wnt receptor signaling pathway
218516_s_at	1.218	2,32624008	myo-inositol monophosphatase A3	IMPA3	8q12.1	---
206201_s_at	1.216	2,32301746	mesenchyme homeo box 2 (growth arrest-specific homeo box)	MEOX2	7p22.1-p21.3	regulation of transcription, DNA-dependent /// development /// circulation
202773_s_at	1.202	2,30058379	splicing factor, arginine/serine-rich 8 (suppressor-of-white-apricot homolog, Drosophila)	SFRS8	12q24.33	nuclear mRNA splicing, via spliceosome /// transcription /// regulation of transcription, DNA-dependent /// mRNA splice site selection
219222_at	1.193	2,28627667	ribokinase	RBKS	2p23.3	carbohydrate metabolism /// D-ribose metabolism
212575_at	1.187	2,27678806	chromosome 19 open reading frame 6	C19orf6	19p13.3	---
211640_x_at	1.151	2,22067767	IgM rheumatoid factor RF-TT1, variable heavy chain /// IgM rheumatoid factor RF-TT1, variable heavy chain	---	---	---
216120_s_at	1.128	2,18555548	ATPase, Ca++ transporting, plasma membrane 2	ATP2B2	3p25.3	cation transport /// calcium ion transport /// metabolism

217081_at	1.127	2,18404109	olfactory receptor, family 2, subfamily H, member 2	OR2H2	6p21.3	regulation of transcription, DNA-dependent /// defense response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// perception of smell /// mating
206720_at	1.094	2,13465068	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	MGAT5	2q21	N-linked glycosylation
217600_at	1.092	2,13169347	Signal peptide, CUB domain, EGF-like 3	SCUBE3	6p21.3	---
210272_at	1.083	2,11843687	cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	CYP2B7P1	19q13.2	electron transport
217104_at	1.057	2,08060053	similar to cervical cancer suppressor-1	LOC400410	15q25.1	---
212338_at	1.028	2,03919537	myosin ID	MYO1D	17q11-q12	---
207894_s_at	1.026	2,0363704	T-cell leukemia/lymphoma 6	TCL6	14q32.1	---
208007_at	1.023	2,03214029	---	---	---	---

Tab. A4b:
Probe-sets up-regulated regulated upon IVMP treatment at day 6 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A4c

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
214660_at	-3.043	-8,24203163	Integrin, alpha 1	PELO	5q11.2	protein biosynthesis
207781_s_at	-2.975	-7,86256479	zinc finger protein 6 (CMPX1)	ZNF6	Xq13-q21.1	regulation of transcription, DNA-dependent /// regulation of transcription
209655_s_at	-2.864	-7,28031059	transmembrane protein 47	TMEM47	Xp11.4	---
221086_s_at	-2.718	-6,57960055	zinc finger protein 312	ZNF312	3p14.2	---
218349_s_at	-2.553	-5,86853338	Zwilch	FLJ10036	15q22.31	---
217400_at	-2.392	-5,24884502	---	---	---	---
215655_at	-2.378	-5,19815625	Glutamate receptor, ionotropic, kainate 2	GRIK2	6q16.3-q21	ion transport /// potassium ion transport /// glutamate signaling pathway /// synaptic transmission /// synaptic transmission
207156_at	-2.321	-4,9967845	histone 1, H2ag	HIST1H2AG	6p22.1	---
216681_at	-2.292	-4,89734557	---	---	---	---
214702_at	-2.26	-4,78991482	fibronectin 1	FN1	2q34	acute-phase response /// cell adhesion /// metabolism /// response to wounding /// cell migration
215047_at	-2.196	-4,58207159	tripartite motif-containing 58	TRIM58	1q44	protein ubiquitination
219937_at	-2.187	-4,55357612	thyrotropin-releasing hormone degrading ectoenzyme	TRHDE	12q15-q21	proteolysis and peptidolysis /// sterol depletion response, sterol regulatory element binding-protein cleavage /// signal transduction /// cell-cell signaling
214078_at	-2.183	-4,54096841	P21 (CDKN1A)-activated kinase 3	PAK3	Xq22.3-q23	protein amino acid phosphorylation
204677_at	-2.168	-4,49399961	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
216180_s_at	-2.11	-4,31691295	synaptojanin 2	SYNJ2	6q25.3	---
205378_s_at	-2.025	-4,06991877	acetylcholinesterase (YT blood group)	ACHE	7q22	acetylcholine catabolism in synaptic cleft /// DNA replication /// cell adhesion /// synaptogenesis /// muscle development /// cell proliferation /// response to wounding /// neurotransmitter catabolism /// amyloid precursor protein metabolism /// positiv
211412_at	-2	-4	peptidyl arginine deiminase, type IV	PADI4	1p36.13	protein modification /// protein modification
219474_at	-1.941	-3,83971704	TPA-induced transmembrane protein	TTMP	3q13.2	---
205532_s_at	-1.922	-3,78948028	cadherin 6, type 2, K-cadherin (fetal kidney)	CDH6	5p15.1-p14	cell adhesion /// homophilic cell adhesion
216991_at	-1.919	-3,78160647	zinc finger protein 224	ZNF224	19q13.2	transcription /// regulation of transcription, DNA-dependent
210261_at	-1.851	-3,60750151	potassium channel, subfamily K, member 2	KCNK2	1q41	ion transport /// potassium ion transport /// potassium ion transport
202837_at	-1.749	-3,38125501	FLN29 gene product	FLN29	12q	---
205188_s_at	-1.736	-3,33110308	SMAD, mothers against DPP homolog 5 (Drosophila)	SMAD5	5q31	transcription /// regulation of transcription, DNA-dependent /// signal transduction /// transforming growth factor beta receptor signaling pathway /// embryonic pattern specification /// BMP signaling pathway
216708_x_at	-1.726	-3,30809347	Immunoglobulin lambda variable 3-21	IGLC2	22q11.2	immune response
204525_at	-1.724	-3,30351066	PHD finger protein 14	PHF14	7p21.3	regulation of transcription, DNA-dependent
213770_at	-1.72	-3,29436407	kinase suppressor of ras	KSR	17q11.2	protein amino acid phosphorylation /// intracellular signaling cascade /// Ras protein signal transduction
222274_at	-1.687	-3,21986455	hypothetical protein FLJ31568	FLJ31568	22q11.23	---
214378_at	-1.687	-3,21986455	---	---	---	---
201911_s_at	-1.646	-3,12964713	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	FARP1	13q32.2	---
219851_at	-1.619	-3,07162054	zinc finger protein 613	ZNF613	19q13.41	regulation of transcription, DNA-dependent
208546_x_at	-1.615	-3,06311599	histone 1, H2bh	HIST1H2BH	6p21.3	nucleosome assembly /// nucleosome assembly /// chromosome organization and biogenesis (sensu Eukaryota)
220061_at	-1.607	-3,04617748	hypothetical protein FLJ20581	FLJ20581	16p12.3	metabolism
214261_s_at	-1.59	-3,01049349	alcohol dehydrogenase 6 (class V)	ADH6	4q23	ethanol oxidation
221397_at	-1.581	-2,99177152	taste receptor, type 2, member 10	TAS2R10	12p13	signal transduction /// G-protein coupled receptor protein signaling pathway /// sensory perception /// perception of taste
205572_at	-1.575	-2,97935493	angiopoietin 2	ANGPT2	8p23.1	angiogenesis /// signal transduction /// cell differentiation
221148_at	-1.546	-2,92006402	---	---	---	---
218748_s_at	-1.545	-2,91804069	SEC10-like 1 (S. cerevisiae)	SEC10L1	14q22.3	exocytosis /// post-Golgi transport /// protein transport /// vesicle docking
204261_s_at	-1.542	-2,9119791	presenilin 2 (Alzheimer disease 4)	PSEN2	1q31-q42	membrane protein ectodomain proteolysis /// chromosome organization and biogenesis (sensu Eukaryota) /// chromosome segregation /// Notch receptor processing /// intracellular signaling cascade /// apoptotic program /// protein processing /// amyloid prec
216079_at	-1.538	-2,90391656	epilepsy, progressive myoclonus type 2A, Lafora disease (laforin)	EPM2A	6q24	carbohydrate metabolism /// glycogen metabolism /// regulation of translation /// protein amino acid dephosphorylation
210476_s_at	-1.523	-2,87388035	prolactin receptor	PRLR	5p14-p13	steroid biosynthesis /// steroid biosynthesis /// anti-apoptosis /// anti-apoptosis /// cell surface receptor linked signal transduction /// cell surface receptor linked signal transduction /// transmembrane receptor protein tyrosine kinase activation (di
220803_at	-1.519	-2,8659233	Associated molecule with the SH3 domain of STAM (AMSH) like protein	AMSH-LP	10q23.31	---
215409_at	-1.497	-2,82255169	PLSC domain containing protein	LOC254531	15q14	metabolism
215767_at	-1.496	-2,82059592	chromosome 2 open reading frame 10	C2orf10	2q32.1	---
220959_s_at	-1.486	-2,80111264	odorant binding protein 2B /// odorant binding protein 2A	OBP2B /// OBP2A	9q34	transport /// perception of smell /// chemosensory behavior /// electron transport /// perception of smell
219387_at	-1.474	-2,77791027	KIAA1212	KIAA1212	2p16.3	---
210896_s_at	-1.471	-2,77213977	aspartate beta-hydroxylase	ASPH	8q12.1	muscle contraction /// peptidyl-amino acid modification
216543_at	-1.471	-2,77213977	Homo sapiens, clone IMAGE:4824772, mRNA	---	---	---
219661_at	-1.442	-2,71697257	RAN binding protein 17	RANBP17	5q34	protein-nucleus import, docking /// protein transport
216468_s_at	-1.438	-2,70944995	zinc finger protein 682	ZNF682	19p12	regulation of transcription, DNA-dependent
211174_s_at	-1.438	-2,70944995	cholecystokinin A receptor	CCKAR	4p15.1-p15.2	smooth muscle contraction /// signal transduction /// positive regulation of cytosolic calcium ion concentration /// response to nutrients /// digestion /// feeding behavior
219887_at	-1.417	-2,67029861	hypothetical protein FLJ10786	FLJ10786	11q13.2	---
217525_at	-1.385	-2,61171957	olfactomedin-like 1	OLFML1	11p15.4	---
210228_at	-1.368	-2,58112498	colony stimulating factor 2 (granulocyte-macrophage)	CSF2	5q31.1	cellular defense response /// cell surface receptor linked signal transduction /// development
221163_s_at	-1.342	-2,53502504	Williams Beuren syndrome chromosome region 14	WBSCR14	7q11.23	transcription /// regulation of transcription, DNA-dependent /// morphogenesis
207019_s_at	-1.337	-2,52625452	A kinase (PRKA) anchor protein 4	AKAP4	Xp11.2	cell motility /// signal transduction /// signal transduction /// fertilization (sensu Metazoa) /// sperm motility
213032_at	-1.32	-2,4966811	Nuclear factor I/B	NFIB	9p24.1	DNA replication /// transcription /// regulation of transcription, DNA-dependent
211812_s_at	-1.313	-2,48457656	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 3	B3GALT3	3q25	protein amino acid glycosylation
221239_s_at	-1.263	-2,39994278	Fc receptor-like 2 /// Fc receptor-like 2	FCRL2	1q21	cell-cell signaling
215695_s_at	-1.244	-2,36854322	glycogenin 2	GYG2	Xp22.3	glycogen biosynthesis /// carbohydrate biosynthesis

209648_x_at	-1.242	-2,385262	suppressor of cytokine signaling 5	SOCS5	2p21	regulation of cell growth /// intracellular signaling cascade /// JAK-STAT cascade /// negative regulation of signal transduction /// cytokine and chemokine mediated signaling pathway /// positive regulation of T-helper 1 cell differentiation /// negative
217082_at	-1.242	-2,385262	Unknown protein	---	---	---
204467_s_at	-1.239	-2,38034869	synuclein, alpha (non A4 component of amyloid precursor) /// synuclein, alpha (non A4 component of amyloid precursor)	SNCA	4q21	anti-apoptosis /// central nervous system development
211520_s_at	-1.221	-2,3310824	glutamate receptor, ionotropic, AMPA 1	GRIA1	5q33|5q31.1	ion transport /// potassium ion transport /// signal transduction /// synaptic transmission
216014_s_at	-1.204	-2,30377528	zinc finger, X-linked, duplicated A /// zinc finger, X-linked, duplicated B	ZXDA /// ZXDB	Xp11.1 /// Xp11.21	---
209135_at	-1.203	-2,30217898	aspartate beta-hydroxylase	ASPH	8q12.1	muscle contraction /// peptidyl-amino acid modification
202684_s_at	-1.195	-2,28944832	RNA (guanine-7-) methyltransferase	RNMT	18p11.22-p11.23	mRNA capping
203220_s_at	-1.177	-2,26106113	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	9q21.32	regulation of transcription, DNA-dependent /// signal transduction /// frizzled signaling pathway /// development /// organogenesis
216023_at	-1.165	-2,24233216	jumonji domain containing 2B	JMJD2B	19p13.3	regulation of transcription, DNA-dependent
205731_s_at	-1.161	-2,2361237	nuclear receptor coactivator 2	NCOA2	8q13.3	transcription /// regulation of transcription, DNA-dependent /// signal transduction
220278_at	-1.132	-2,19162353	jumonji domain containing 2D	JMJD2D	11q21	---
205980_s_at	-1.126	-2,18252775	Rho GTPase activating protein 8	ARHGAP8	22q13.31	---
216392_s_at	-1.119	-2,17196371	SEC23 interacting protein	SEC23IP	10q25-q26	intracellular protein transport /// Golgi organization and biogenesis
214831_at	-1.117	-2,16895482	Mediator of RNA polymerase II transcription, subunit 28 homolog (yeast)	MED28	4p16	transcription /// regulation of transcription, DNA-dependent
206024_at	-1.101	-2,14503323	4-hydroxyphenylpyruvate dioxygenase	HPD	12q24-qter	L-phenylalanine catabolism /// tyrosine catabolism /// aromatic amino acid family metabolism
217081_at	-1.101	-2,14503323	olfactory receptor, family 2, subfamily H, member 2	OR2H2	6p21.3	regulation of transcription, DNA-dependent /// defense response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// perception of smell /// mating
200690_at	-1.089	-2,12726535	heat shock 70kDa protein 9B (mortalin-2)	HSPA9B	5q31.1	protein folding
215481_s_at	-1.084	-2,11990557	peroxisomal biogenesis factor 5	PEX5	12p13.3	protein transport
211813_x_at	-1.073	-2,10380356	decorin	DCN	12q21.33	organogenesis
214612_x_at	-1.038	-2,05337909	melanoma antigen family A, 6	MAGEA6	Xq28	---
217687_at	-1.03	-2,04202425	adenylate cyclase 2 (brain)	ADCY2	5p15.3	cAMP biosynthesis /// intracellular signaling cascade /// cyclic nucleotide biosynthesis
215034_s_at	-1.029	-2,04060932	transmembrane 4 L six family member 1	TM4SF1	3q21-q25	---
37953_s_at	-1.027	-2,03778239	amiloride-sensitive cation channel 2, neuronal	ACCN2	12q12	ion transport /// sodium ion transport /// signal transduction /// response to pH
209981_at	-1.024	-2,03354935	RNA-binding protein pippin	PIPPIN	22q13.2-q13.31	regulation of transcription, DNA-dependent /// mRNA processing /// histone mRNA 3'-end processing
220790_s_at	-1.02	-2,02791896	membrane-spanning 4-domains, subfamily A, member 5	MS4A5	11q12	signal transduction
213597_s_at	-1.017	-2,0237084	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	CTDSPL	3p21.3	---

Tab. A4c:
Probe-sets down-regulated regulated upon IVMP treatment at day 21 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A4d

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosome Location	GO Biological Process Description
207142_at	2.52	5,74	potassium inwardly-rectifying channel, subfamily J, member 3	KCNJ3	2q24.1	ion transport /// potassium ion transport
220837_at	2.518	5,73	---	---	---	---
205973_at	2.411	5,32	fasciculation and elongation protein zeta 1 (zygin I)	FEZ1	11q24.2	cell adhesion /// neurogenesis /// axon guidance
203324_s_at	2.384	5,22	caveolin 2	CAV2	7q31.1	---
208571_at	2.253	4,77	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A /// acidic (leucine-rich) nuclear phosphoprotein 32 family, member D /// acidic (leucine-rich) nuclear phosphoprotein 32 family, member C /// similar to acidic (leucine-rich) nuclear phospho	ANP32A /// ANP32D /// ANP32C /// LOC440272	15q22.3-q23 /// 12q13.11 /// 4q32.3 /// 15q14	nucleocytoplasmic transport /// intracellular signaling cascade
207118_s_at	2.221	4,66	matrix metalloproteinase 23B /// matrix metalloproteinase 23A	MMP23B /// MMP23A	1p36.3	reproduction /// proteolysis and peptidolysis /// proteolysis and peptidolysis
214981_at	2.152	4,44	Periostin, osteoblast specific factor	POSTN	13q13.3	skeletal development /// cell adhesion /// cell adhesion
212575_at	2.105	4,30	chromosome 19 open reading frame 6	C19orf6	19p13.3	---
220090_at	2.042	4,12	chromosome 1 open reading frame 10	C1orf10	1q21	response to unfolded protein /// metabolism /// response to heat /// cell-cell adhesion /// cell-cell adhesion
217196_s_at	2.034	4,10	KIAA1078 protein	KIAA1078	1q32.1	---
215324_at	2.026	4,07	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	SEMA3D	7q21.11	neurogenesis /// cell differentiation
206201_s_at	1.961	3,89	mesenchyme homeo box 2 (growth arrest-specific homeo box)	MEOX2	7p22.1-p21.3	regulation of transcription, DNA-dependent /// development /// circulation
216155_at	1.906	3,75	Neuron navigator 1	NAV1	---	DNA methylation
205697_at	1.821	3,53	secretagogin, EF-hand calcium binding protein	SCGN	6p22.3-p22.1	---
222363_at	1.815	3,52	Transcribed locus	---	---	---
204696_s_at	1.799	3,48	cell division cycle 25A	CDC25A	3p21	regulation of cyclin dependent protein kinase activity /// cytokinesis /// protein amino acid dephosphorylation /// mitosis /// cell proliferation
218719_s_at	1.795	3,47	hypothetical protein FLJ13912	FLJ13912	16q21	---
220909_at	1.788	3,45	tripartite motif-containing 46	TRIM46	1q22	protein ubiquitination
217612_at	1.784	3,44	translocase of inner mitochondrial membrane 50 homolog (yeast)	TIMM50	19q13.2	---
215408_at	1.779	3,43	Myosin IE	MYO1E	15q21-q22	actin filament-based movement
212338_at	1.777	3,43	myosin ID	MYO1D	17q11-q12	---
205593_s_at	1.744	3,35	phosphodiesterase 9A	PDE9A	21q22.3	signal transduction /// signal transduction
201621_at	1.723	3,30	neuroblastoma, suppression of tumorigenicity 1	NBL1	1p36.13-p36.11	cell cycle /// negative regulation of cell cycle
213591_at	1.676	3,20	aldehyde dehydrogenase 7 family, member A1	ALDH7A1	5q31	aldehyde metabolism /// perception of sound /// metabolism
204433_s_at	1.639	3,11	spermatogenesis associated 2	SPATA2	20q13.1-q13.2	spermatogenesis /// cell differentiation
213951_s_at	1.624	3,08	TBP-1 interacting protein	TBPIP	17q12-q21	---
219082_at	1.621	3,08	CGI-14 protein	CGI-14	16p13.3	N-acetylglucosamine metabolism
206295_at	1.621	3,08	interleukin 18 (interferon-gamma-inducing factor)	IL18	11q22.2-q22.3	angiogenesis /// immune response /// cell-cell signaling /// induction of apoptosis via death domain receptors /// regulation of cell adhesion /// sleep /// chemokine biosynthesis /// T-helper 2 type immune response /// interleukin-2 biosynthesis /// inte
205535_s_at	1.6	3,03	BH-protocadherin (brain-heart)	PCDH7	4p15	cell adhesion /// homophilic cell adhesion
204726_at	1.594	3,02	cadherin 13, H-cadherin (heart)	CDH13	16q24.2-q24.3	cell adhesion /// homophilic cell adhesion
212942_s_at	1.566	2,98	KIAA1199	KIAA1199	15q24	perception of sound
205659_at	1.538	2,90	histone deacetylase 9	HDAC9	7p21.1	regulation of cell cycle /// transcription /// regulation of transcription, DNA-dependent /// inflammatory response /// chromatin modification /// histone deacetylation /// B-cell differentiation /// negative regulation of myogenesis
211786_at	1.536	2,90	tumor necrosis factor receptor superfamily, member 9 /// tumor necrosis factor receptor superfamily, member 9	TNFRSF9	1p36	induction of apoptosis /// immune response /// negative regulation of cell proliferation
221350_at	1.519	2,87	homeo box C8	HOXC8	12q13.3	regulation of transcription, DNA-dependent /// development
220504_at	1.498	2,82	keratocan	KERA	12q22	eye morphogenesis (sensu Mammalia) /// sensory perception /// visual perception
219878_s_at	1.484	2,80	Kruppel-like factor 13	KLF13	15q12	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter
210852_s_at	1.484	2,80	aminoadipate-semialdehyde synthase	AASS	7q31.3	electron transport /// lysine catabolism /// protein tetramerization
207645_s_at	1.48	2,79	chromodomain helicase DNA binding protein 1-like	CHD1L	1q12	---
221577_x_at	1.477	2,78	growth differentiation factor 15	GDF15	19p13.1-13.2	signal transduction /// transforming growth factor beta receptor signaling pathway /// cell cell signaling
203295_s_at	1.476	2,78	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide	ATP1A2	1q21-q23	potassium ion transport /// sodium ion transport /// metabolism /// ATP hydrolysis coupled proton transport /// sperm motility /// hydrogen ion homeostasis
213991_s_at	1.473	2,78	Heparan sulfate (glucosamine) 3 O-sulfotransferase 1	HS3ST1	4p16	---
205085_at	1.406	2,65	origin recognition complex, subunit 1-like (yeast)	ORC1L	1p32	DNA replication /// DNA replication initiation
220830_at	1.401	2,64	interphotoreceptor matrix proteoglycan 2	IMPG2	3q12.2-q12.3	visual perception
219932_at	1.381	2,60	solute carrier family 27 (fatty acid transporter), member 6	SLC27A6	5q23.3	very-long-chain fatty acid metabolism /// lipid metabolism /// metabolism
207484_s_at	1.368	2,58	HLA-B associated transcript 8	BAT8	6p21.31	chromatin modification
215139_at	1.346	2,54	Rho guanine nucleotide exchange factor (GEF) 10	ARHGEF10	8p23	---

201452_at	1.342	2,54	Ras homolog enriched in brain	RHEB	7q36	signal transduction /// small GTPase mediated signal transduction
214418_at	1.336	2,52	hypothetical protein LOC196993	LOC196993	15q22.32	---
205990_s_at	1.333	2,52	wingless-type MMTV integration site family, member 5A	WNT5A	3p21-p14	signal transduction /// frizzled-2 signaling pathway /// cell-cell signaling /// morphogenesis
203918_at	1.333	2,52	protocadherin 1 (cadherin-like 1)	PCDH1	5q32-q33	regulation of transcription, DNA-dependent /// cell adhesion /// homophilic cell adhesion /// cell-cell signaling /// neurogenesis
203862_s_at	1.332	2,52	actinin, alpha 2	ACTN2	1q42-q43	---
211347_at	1.324	2,50	CDC14 cell division cycle 14 homolog B (S. cerevisiae)	CDC14B	9q22.33	protein amino acid dephosphorylation
210610_at	1.319	2,49	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	CEACAM1	19q13.2	immune response /// pregnancy
215712_s_at	1.298	2,46	insulin-like growth factor binding protein, acid labile subunit	IGFALS	16p13.3	cell adhesion /// signal transduction
207894_s_at	1.287	2,44	T-cell leukemia/lymphoma 6	TCL6	14q32.1	---
207754_at	1.279	2,43	chromosome 12 open reading frame 2	C12orf2	12p12.3	signal transduction
217616_at	1.268	2,41	SNF1-like kinase	SNF1LK	21q22.3	protein amino acid phosphorylation /// cell cycle /// protein kinase cascade /// regulation of mitotic cell cycle /// regulation of cell differentiation
205151_s_at	1.26	2,39	KIAA0644 gene product	KIAA0644	7p15.1	---
217032_at	1.254	2,39	forkhead box D4 like 1	FOXD4L1	2q14.1	transcription /// regulation of transcription, DNA-dependent
40489_at	1.245	2,37	atrophin 1	ATN1	12p13.31	central nervous system development
216214_at	1.225	2,34	Clone 24504 mRNA sequence	---	---	---
221674_s_at	1.212	2,32	chordin	CHRD	3q27	skeletal development /// development
216245_at	1.193	2,29	interleukin 1 receptor antagonist	IL1RN	2q14.2	inflammatory response
219832_s_at	1.176	2,26	homeo box C13	HOXC13	12q13.3	regulation of transcription, DNA-dependent /// morphogenesis
53202_at	1.176	2,26	chromosome 7 open reading frame 25	C7orf25	7p14-p11.2	---
209803_s_at	1.164	2,24	pleckstrin homology-like domain, family A, member 2	PHLDA2	11p15.5	imprinting /// apoptosis
217123_x_at	1.16	2,23	pro-melanin-concentrating hormone-like 1	PMCHL1	5p14.3	synaptic transmission /// behavior
212923_s_at	1.154	2,23	chromosome 6 open reading frame 145	C6orf145	6p25.2	---
217625_x_at	1.143	2,21	Hypothetical gene supported by AK024177	---	9q34.11	---
221863_at	1.125	2,18	KIAA1193	KIAA1193	19p13.3	---
216017_s_at	1.103	2,15	NGFI-A binding protein 2 (EGR1 binding protein 2)	NAB2	12q13.3-q14.1	transcription /// regulation of transcription, DNA-dependent /// neurogenesis /// cell proliferation /// negative regulation of transcription
220638_s_at	1.098	2,14	Cas-Br-M (murine) ecotropic retroviral transforming sequence c	CBLC	19q13.2	---
217633_at	1.095	2,14	chromosome 21 open reading frame 108	C21orf108	21q22.11	---
216690_at	1.062	2,09	olfactory receptor, family 7, subfamily C, member 1	OR7C1	19p13.1	---
209373_at	1.055	2,08	BENE protein	BENE	2q13	---
213772_s_at	1.047	2,07	golgi associated, gamma adaptin ear containing, ARF binding protein 2	GGA2	16p12	protein complex assembly /// intracellular protein transport /// intracellular protein transport /// intra-Golgi transport
219534_x_at	1.024	2,03	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
213997_at	1.008	2,01	KIAA0574 protein	KIAA0574	15q12	---

Tab. A4d:
Probe-sets up-regulated regulated upon IVMP treatment at day 21 compared to day 0;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A4e

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosoma Location	GO Biological Process Description
219937_at	-1.021	-2,02932509	thyrotropin-releasing hormone degrading ectoenzyme	TRHDE	12q15-q21	proteolysis and peptidolysis /// sterol depletion response, sterol regulatory element binding-protein cleavage /// signal transduction /// cell-cell signaling
203862_s_at	-1.027	-2,03778239	actinin, alpha 2	ACTN2	1q42-q43	---
215655_at	-1.05	-2,07052985	Glutamate receptor, ionotropic, kainate 2	GRIK2	6q16.3-q21	ion transport /// potassium ion transport /// glutamate signaling pathway /// synaptic transmission /// synaptic transmission
215107_s_at	-1.054	-2,07827854	hypothetical protein FLJ20619	FLJ20619	1p32.3	---
219537_x_at	-1.058	-2,0820432	delta-like 3 (Drosophila)	DLL3	19q13	skeletal development /// embryonic development (sensu Mammalia) /// cell fate determination /// Notch signaling pathway /// Notch signaling pathway /// development /// neurogenesis /// cell differentiation
219222_at	-1.082	-2,11696879	ribokinase	RBKS	2p23.3	carbohydrate metabolism /// D-ribose metabolism
214660_at	-1.086	-2,12284642	Integrin, alpha 1	PELO	5q11.2	protein biosynthesis
209101_at	-1.09	-2,12874036	connective tissue growth factor	CTGF	6q23.1	regulation of cell growth /// DNA metabolism /// cell motility /// cell adhesion /// epidermis development /// response to wounding
213032_at	-1.122	-2,17648488	Nuclear factor I/B	NFIB	9p24.1	DNA replication /// transcription /// regulation of transcription, DNA-dependent
204677_at	-1.137	-2,1992323	cadherin 5, type 2, VE-cadherin (vascular epithelium)	CDH5	16q22.1	cell adhesion /// homophilic cell adhesion
210871_x_at	-1.14	-2,20381023	synovial sarcoma, X breakpoint 2 interacting protein	SSX2IP	1p22.3	cell adhesion
211584_s_at	-1.142	-2,20686748	nuclear protein, ataxia-telangiectasia locus	NPAT	11q22-q23	---
205731_s_at	-1.15	-2,21913894	nuclear receptor coactivator 2	NCOA2	8q13.3	transcription /// regulation of transcription, DNA-dependent /// signal transduction
209353_s_at	-1.156	-2,2283873	hypothetical protein MGC16664	MGC16664	1q25.2	---
214668_at	-1.164	-2,24077843	chromosome 13 open reading frame 1	C13orf1	13q14	---
206783_at	-1.164	-2,24077843	fibroblast growth factor 4 (heparin secretory transforming protein 1, Kaposi sarcoma oncogene)	FGF4	11q13.3	regulation of cell cycle /// mitosis /// signal transduction /// cell-cell signaling /// cell proliferation /// positive regulation of cell proliferation
222374_at	-1.166	-2,24388696	beta-transducin repeat containing	BTRC	10q24.32	ubiquitin-dependent protein catabolism /// ubiquitin cycle /// signal transduction /// Wnt receptor signaling pathway
206480_at	-1.175	-2,25792881	leukotriene C4 synthase	LTC4S	5q35	leukotriene biosynthesis
220815_at	-1.184	-2,27205853	catenin (cadherin-associated protein), alpha 3	CTNNA3	10q22.2	cell adhesion
213381_at	-1.202	-2,30058379	Chromosome 10 open reading frame 72	C10orf72	10q11.23	---
210272_at	-1.21	-2,31337637	cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	CYP2B7P1	19q13.2	electron transport
208546_x_at	-1.216	-2,32301748	histone 1, H2bh	HIST1H2BH	6p21.3	nucleosome assembly /// nucleosome assembly /// chromosome organization and biogenesis (sensu Eukaryota)
202773_s_at	-1.249	-2,37676821	splicing factor, arginine/serine-rich 8 (suppressor-of-white-apricot homolog, Drosophila)	SFRS8	12q24.33	nuclear mRNA splicing, via spliceosome /// transcription /// regulation of transcription, DNA-dependent /// mRNA splice site selection
221180_at	-1.289	-2,4435882	regulated in COPD kinase	RCK	2q21.3	protein amino acid phosphorylation
213597_s_at	-1.364	-2,57397849	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	CTDSPL	3p21.3	---
220360_at	-1.366	-2,57754926	THAP domain containing 9	THAP9	4q21.3	---
219474_at	-1.373	-2,590088	TPA-induced transmembrane protein	TTMP	3q13.2	---
205290_s_at	-1.375	-2,59367911	bone morphogenetic protein 2	BMP2	20p12	skeletal development /// cell-cell signaling /// cell differentiation /// growth
220843_s_at	-1.376	-2,59547753	gene model 83	Gm83	8q22.3	---
210148_at	-1.402	-2,64267681	homeodomain interacting protein kinase 3	HIPK3	11p13	transcription /// regulation of transcription, DNA-dependent /// protein amino acid phosphorylation /// apoptosis
205188_s_at	-1.422	-2,6795672	SMAD, mothers against DPP homolog 5 (Drosophila)	SMAD5	5q31	transcription /// regulation of transcription, DNA-dependent /// signal transduction /// transforming growth factor beta receptor signaling pathway /// embryonic pattern specification /// BMP signaling pathway
206537_at	-1.423	-2,68142518	---	---	---	---
37953_s_at	-1.445	-2,72262823	amiloride-sensitive cation channel 2, neuronal	ACCN2	12q12	ion transport /// sodium ion transport /// signal transduction /// response to pH
217565_at	-1.449	-2,73018744	glutamate receptor, ionotrophic, AMPA 3	GRIA3	Xq25-q26	ion transport /// potassium ion transport /// glutamate signaling pathway /// synaptic transmission
203797_at	-1.453	-2,73778763	visinin-like 1	VSNL1	2p24.3	---
220425_x_at	-1.46	-2,75108384	ropporin, rhophilin associated protein 1B	ROPN1B	3q21.2	cytokinesis /// signal transduction /// Rho protein signal transduction /// spermatogenesis /// acrosome reaction /// fusion of sperm to egg plasma membrane /// cell-cell adhesion /// sperm motility
205385_at	-1.471	-2,77213977	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	MDM2	12q14.3-q15	regulation of cell cycle /// negative regulation of transcription from RNA polymerase II promoter /// negative regulation of transcription from RNA polymerase II promoter /// protein complex assembly /// negative regulation of cell proliferation /// prote
217600_at	-1.475	-2,77983844	Signal peptide, CUB domain, EGF-like 3	SCUBE3	6p21.3	---

210674_s_at	-1.484	-2.79723217	protocadherin alpha 9 /// protocadherin alpha subfamily C, 2 /// protocadherin alpha subfamily C, 1 /// protocadherin alpha 13 /// protocadherin alpha 12 /// protocadherin alpha 11 /// protocadherin alpha 10 /// protocadherin alpha 8 /// protocadherin alp	PCDHAC2 /// PCDHAC1 /// PCDHA13 /// PCDHA12 /// PCDHA11 /// PCDHA10 /// PCDHA8 /// PCDHA7 /// PCDHA6 /// PCDHA5 /// PCDHA4 /// PCDHA3 /// PCDHA2 /// PCDHA1	5q31	cell adhesion /// homophilic cell adhesion /// cell adhesion /// neurogenesis
217589_at	-1.493	-2.81473675	RAB40A, member RAS oncogene family	RAB40A	Xq22.1	intracellular protein transport /// small GTPase mediated signal transduction /// protein transport
213770_at	-1.509	-2.84612692	kinase suppressor of ras	KSR	17q11.2	protein amino acid phosphorylation /// intracellular signaling cascade /// Ras protein signal transduction
210476_s_at	-1.516	-2.85996997	prolactin receptor	PRLR	5p14-p13	steroid biosynthesis /// steroid biosynthesis /// anti-apoptosis /// cell surface receptor linked signal transduction /// cell surface receptor linked signal transduction /// transmembrane receptor protein tyrosine kinase activation (di
206898_at	-1.518	-2.86393748	cadherin 19, type 2	CDH19	18q22-q23	homophilic cell adhesion
205791_x_at	-1.524	-2.87587307	zinc finger protein 230	ZNF230	19q13.31	transcription /// regulation of transcription, DNA-dependent
217104_at	-1.545	-2.91804069	similar to cervical cancer suppressor 1	LOC400410	15q25.1	---
206175_x_at	-1.559	-2.94849537	zinc finger protein 222	ZNF222	19q13.2	transcription /// regulation of transcription, DNA-dependent
208329_at	-1.567	-2.9628796	prostate and breast cancer overexpressed 1	PBOV1	6q23-q24	---
215746_at	-1.58	-2.9896985	Tetracycline transporter-like protein	TETRAN	4p16.3	transport
216392_s_at	-1.609	-3.05040331	SEC23 interacting protein	SEC23IP	10q25-q26	intracellular protein transport /// Golgi organization and biogenesis
214612_x_at	-1.616	-3.06523992	melanoma antigen family A, 6	MAGEA6	Xq28	---
208164_s_at	-1.623	-3.0801487	interleukin 9 receptor /// similar to interleukin 9 receptor	IL9R /// LOC400481	Xq28 and Yq12 /// 16p13.3	signal transduction /// cell proliferation
207256_at	-1.66	-3.16016525	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	MBL2	10q11.2-q21	complement activation, lectin pathway /// phosphate transport /// immune response /// complement activation, classical pathway /// response to oxidative stress
211640_x_at	-1.663	-3.16674346	IgM rheumatoid factor RF-TT1, variable heavy chain /// IgM rheumatoid factor RF-TT1, variable heavy chain	---	---	---
215034_s_at	-1.669	-3.179941	transmembrane 4 L six family member 1	TM4SF1	3q21-q25	---
208007_at	-1.676	-3.19540787	---	---	---	---
207437_at	-1.687	-3.21986455	neuro-oncological ventral antigen 1	NOVA1	14q	synaptic transmission /// synaptic transmission /// locomotory behavior /// locomotory behavior /// RNA splicing /// RNA splicing
209648_x_at	-1.74	-3.34035168	suppressor of cytokine signaling 5	SOCS5	2p21	regulation of cell growth /// intracellular signaling cascade /// JAK-STAT cascade /// negative regulation of signal transduction /// cytokine and chemokine mediated signaling pathway /// positive regulation of T-helper 1 cell differentiation /// negative
207192_at	-1.744	-3.34962595	deoxyribonuclease I-like 2	DNASE1L2	16p13.3	DNA catabolism
205925_s_at	-1.75	-3.36358566	RAB3B, member RAS oncogene family	RAB3B	1p32-p31	small GTPase mediated signal transduction /// protein transport
210896_s_at	-1.761	-3.38932974	aspartate beta-hydroxylase	ASPH	8q12.1	muscle contraction /// peptidyl-amino acid modification
216427_at	-1.765	-3.39874	CDNA: FLJ22786 fis, clone KAIA2150	---	---	---
218748_s_at	-1.78	-3.43426175	SEC10-like 1 (S. cerevisiae)	SEC10L1	14q22.3	exocytosis /// post-Golgi transport /// protein transport /// vesicle docking
207846_at	-1.781	-3.43664302	POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	POU1F1	3p11	regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// negative regulation of cell proliferation /// organogenesis
206720_at	-1.788	-3.45335823	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	MGAT5	2q21	N-linked glycosylation
81737_at	-1.796	-3.47256091	Similar to Group X secretory phospholipase A2 precursor (Phosphatidylcholine 2-acylhydrolase GX) (GX sPLA2) (sPLA2-X)	---	16p13.11	---
219887_at	-1.808	-3.50156532	hypothetical protein FLJ10786	FLJ10786	11q13.2	---
204261_s_at	-1.818	-3.52592063	presenilin 2 (Alzheimer disease 4)	PSEN2	1q31-q42	membrane protein ectodomain proteolysis /// chromosome organization and biogenesis (sensu Eukaryota) /// chromosome segregation /// Notch receptor processing /// intracellular signaling cascade /// apoptotic program /// protein processing /// amyloid prec
214375_at	-1.872	-3.66039667	PTPRF interacting protein, binding protein 1 (liprin beta 1) /// similar to PTPRF interacting protein binding protein 1 isoform 1; liprin-beta 1; liprin related protein; protein-tyrosine phosphatase receptor-type f polypeptide-interacting protein-binding	PPFIBP1 /// LOC440091	12p11.23-p11.22 /// 12p11.23	cell adhesion
216120_s_at	-1.899	-3.72954595	ATPase, Ca++ transporting, plasma membrane 2	ATP2B2	3p25.3	cation transport /// calcium ion transport /// metabolism
205572_at	-1.947	-3.85571923	angiopoietin 2	ANGPT2	8p23.1	angiogenesis /// signal transduction /// cell differentiation
219945_at	-2.001	-4.00277355	DEAD (Asp-Glu-Ala-Asp) box polypeptide 25	DDX25	11q24	---

221163_s_at	-2.052	-4,1468044	Williams Beuren syndrome chromosome region 14	WBSCR14	7q11.23	transcription /// regulation of transcription, DNA-dependent /// morphogenesis
214831_at	-2.068	-4,1930499	Mediator of RNA polymerase II transcription, subunit 28 homolog (yeast)	MED28	4p16	transcription /// regulation of transcription, DNA-dependent
220278_at	-2.074	-4,21052482	jumonji domain containing 2D	JMJD2D	11q21	---
215944_at	-2.125	-4,38203093	---	---	---	---
214078_at	-2.159	-4,48605185	P21 (CDKN1A)-activated kinase 3	PAK3	Xq22.3-q23	protein amino acid phosphorylation
219872_at	-2.164	-4,48155688	hypothetical protein DKFZp434L142	DKFZp434L142	4q32.1	---
205532_s_at	-2.194	-4,57572389	cadherin 6, type 2, K-cadherin (fetal kidney)	CDH6	5p15.1-p14	cell adhesion /// homophilic cell adhesion
216814_at	-2.205	-4,61074539	---	---	---	---
217081_at	-2.228	-4,68484072	olfactory receptor, family 2, subfamily H, member 2	OR2H2	6p21.3	regulation of transcription, DNA-dependent /// defense response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// perception of smell /// mating
205033_s_at	-2.371	-5,17298573	defensin, alpha 1, myeloid-related sequence /// defensin, alpha 3, neutrophil-specific	DEFA1 /// DEFA3	8p23.1 /// 8pter-p23.3	xenobiotic metabolism /// response to pest, pathogen or parasite /// defense response to bacteria /// defense response to fungi
215073_s_at	-2.425	-5,37029001	nuclear receptor subfamily 2, group F, member 2	NR2F2	15q26	transcription /// regulation of transcription from RNA polymerase II promoter /// lipid metabolism /// signal transduction
204286_s_at	-2.868	-7,30052391	phorbol-12-myristate-13-acetate-induced protein 1	PMAIP1	18q21.32	---
209298_s_at	-2.88	-7,3815012	intersectin 1 (SH3 domain protein)	ITSN1	21q22.1-q22.2	synaptic vesicle endocytosis
218349_s_at	-3.187	-9,10715223	Zwilch	FLJ10036	15q22.31	---

Tab. A4e:
Probe-sets down-regulated regulated upon IVMP treatment at day 21 compared to day 6;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A4f

Probe Set ID	log2 FC	Fold Change	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
220798_x_at	1.401	2,64084568	plasticity-related gene 2	PRG2	19p13.3	---
205533_s_at	1.158	2,23147864	cadherin 6, type 2, K-cadherin (fetal kidney)	CDH6	5p15.1-p14	cell adhesion /// homophilic cell adhesion
209803_s_at	1.802	3,48703296	pleckstrin homology-like domain, family A, member 2	PHLDA2	11p15.5	imprinting /// apoptosis
201741_x_at	1.235	2,35381347	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	SFRS1	17q21.3-q22	nuclear mRNA splicing, via spliceosome /// mRNA splice site selection
208062_s_at	1.305	2,47083727	neuregulin 2	NRG2	5q23-q33	anti-apoptosis /// signal transduction /// cell-cell signaling /// embryonic development
205990_s_at	3.111	8,63981248	wingless-type MMTV integration site family, member 5A	WNT5A	3p21-p14	signal transduction /// frizzled-2 signaling pathway /// cell-cell signaling /// morphogenesis
220830_at	1.15	2,21913894	interphotoreceptor matrix proteoglycan 2	IMPG2	3q12.2-q12.3	visual perception
209544_at	1.576	2,98142077	receptor-interacting serine-threonine kinase 2	RIPK2	8q21	protein amino acid phosphorylation /// inflammatory response /// signal transduction /// regulation of apoptosis /// positive regulation of I-kappaB kinase/NF-kappaB cascade
213951_s_at	1.144	2,20992897	TBP-1 interacting protein	TBPIP	17q12-q21	---
220200_s_at	1.698	3,24450862	PR/SET domain containing protein 8	SET8	12q24.31	chromatin modification
206148_at	1.416	2,66844634	interleukin 3 receptor, alpha (low affinity)	IL3RA	Xp22.3 or Yp11.3	protein amino acid phosphorylation /// development
206742_at	1.306	2,47255052	c-fos induced growth factor (vascular endothelial growth factor D)	FIGF	Xp22.31	regulation of cell cycle /// angiogenesis /// cell proliferation /// positive regulation of cell proliferation /// cell differentiation
207510_at	1.372	2,58829131	bradykinin receptor B1	BDKRB1	14q32.1-q32.2	inflammatory response /// signal transduction /// G-protein coupled receptor protein signaling pathway /// positive regulation of cytosolic calcium ion concentration
218110_at	1.11	2,15845647	XPA binding protein 2	XAB2	19p13.2	DNA repair /// transcription-coupled nucleotide-excision repair /// transcription /// RNA processing
213395_at	1.305	2,47083727	megalencephalic leukoencephalopathy with subcortical cysts 1	MLC1	22q13.33	protein biosynthesis /// ion transport
209269_s_at	1.185	2,27363395	Spleen tyrosine kinase	SYK	9q22	protein complex assembly /// protein amino acid phosphorylation /// leukocyte cell adhesion /// integrin-mediated signaling pathway /// intracellular signaling cascade /// cell proliferation /// organogenesis /// neutrophil chemotaxis
222025_s_at	1.684	3,21317599	5-oxoprolinase (ATP-hydrolysing)	OPLAH	8q24.3	---
213182_x_at	2.21	4,62675274	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
207754_at	1.998	3,99445866	chromosome 12 open reading frame 2	C12orf2	12p12.3	signal transduction
220903_at	2.143	4,41879539	G elongation factor, mitochondrial 1	GFM1	3q25.1-q26.2	protein biosynthesis /// translational elongation
222172_at	1.107	2,15397275	neuronal PAS domain protein 3	NPAS3	14q12-q13	transcription /// regulation of transcription, DNA-dependent /// signal transduction
212923_s_at	1.428	2,69073442	chromosome 6 open reading frame 145	C6orf145	6p25.2	---
216613_at	1.664	3,16893924	MRNA; cDNA DKFZp566L0824 (from clone DKFZp566L0824)	---	---	---
205659_at	1.229	2,34404457	histone deacetylase 9	HDAC9	7p21.1	regulation of cell cycle /// transcription /// regulation of transcription, DNA-dependent /// inflammatory response /// chromatin modification /// histone deacetylation /// B-cell differentiation /// negative regulation of myogenesis
210164_at	2.083	4,23687334	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) /// granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB	14q11.2	proteolysis and peptidolysis /// apoptosis /// cleavage of lamin /// cytolysis
204057_at	1.412	2,66105808	interferon regulatory factor 8 /// interferon regulatory factor 8	IRF8	16q24.1	negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// immune response
215139_at	1.26	2,39495741	Rho guanine nucleotide exchange factor (GEF) 10	ARHGEF10	8p23	---
213966_at	1.596	3,02303986	High-mobility group 20B	HMG20B	19p13.3	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// cell cycle /// chromatin modification
211381_x_at	1.134	2,19466388	sperm associated antigen 11	SPAG11	8p23-p22	defense response /// spermatogenesis /// response to pest, pathogen or parasite
211914_x_at	1.508	2,84415482	neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease) /// neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	NF1	17q11.2	cell cycle /// Ras protein signal transduction /// negative regulation of cell proliferation /// negative regulation of cell cycle
206981_at	1.19	2,28152743	sodium channel, voltage-gated, type IV, alpha	SCN4A	17q23-q25.3	cation transport /// sodium ion transport /// muscle contraction
210415_s_at	1.149	2,21760129	outer dense fiber of sperm tails 2	ODF2	9q34.11	---
216655_s_at	1.737	3,33341283	---	---	---	---
221705_s_at	1.095	2,13813082	hypothetical protein FLJ21168 /// hypothetical protein FLJ21168	FLJ21168	1p13.1	---
201621_at	1.387	2,5793385	neuroblastoma, suppression of tumorigenicity 1	NBL1	1p36.13-p36.11	cell cycle /// negative regulation of cell cycle
222298_at	2.082	4,23393758	Coatomer protein complex, subunit gamma 2	COPG2	7q32	retrograde transport, Golgi to ER /// intra-Golgi transport /// protein transport
209819_at	1.438	2,70944995	hyaluronan binding protein 4	HABP4	9q22.3-q31	---

208571_at	1.578	2,98555677	basic (leucine-rich) nuclear phosphoprotein 32 family, member A /// acidic (leucine-rich) nuclear phosphoprotein 32 family, member D /// acidic (leucine-rich) nuclear phosphoprotein 32 family, member C /// similar to acidic (leucine-rich) nuclear phospho	ANP32A /// ANP32D /// ANP32C /// LOC440272	15q22.3-q23 /// 12q13.11 /// 4q32.3 /// 15q14	nucleocytoplasmic transport /// intracellular signaling cascade
207481_at	2.143	4,41679539	---	---	---	---
217276_x_at	1.623	3,0801487	serine hydrolase-like 2	SERHL2	22q13	aromatic compound metabolism
215712_s_at	1.146	2,21299471	insulin-like growth factor binding protein, acid labile subunit	IGFALS	16p13.3	cell adhesion /// signal transduction
206065_s_at	1.948	3,85304758	dihydropyrimidinase	DPYS	8q22	nucleobase, nucleoside, nucleotide and nucleic acid metabolism /// response to toxin
205898_at	1.809	3,50399326	chemokine (C-X3-C motif) receptor 1	CX3CR1	3p21|3p21.3	chemotaxis /// cellular defense response /// cell adhesion /// signal transduction /// G-protein coupled receptor protein signaling pathway
215408_at	1.103	2,14800894	Myosin IE	MYO1E	15q21-q22	actin filament-based movement
207645_s_at	1.759	3,38463439	chromodomain helicase DNA binding protein 1-like	CHD1L	1q12	---
210726_at	1.682	3,20872467	cytochrome P450, family 3, subfamily A, polypeptide 4	CYP3A4	7q21.1	electron transport /// lipid metabolism /// xenobiotic metabolism /// xenobiotic metabolism /// transport
216644_at	1.304	2,46912521	CDNA FLJ20178 fis, clone COL09990	---	---	---
204607_at	1.695	3,23776887	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	HMGCS2	1p13-p12	acetyl-CoA metabolism /// cholesterol biosynthesis
219878_s_at	1.603	3,03774338	Kruppel-like factor 13	KLF13	15q12	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter
217612_at	1.124	2,17950422	translocase of inner mitochondrial membrane 50 homolog (yeast)	TIMM50	19q13.2	---
206637_at	1.997	3,99169088	purinergic receptor P2Y, G-protein coupled, 14	P2RY14	3q21-q25	signal transduction /// G-protein coupled receptor protein signaling pathway
204753_s_at	1.777	3,42712782	hepatic leukemia factor	HLF	17q22	transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// development /// rhythmic process
203163_at	1.04	2,05622765	katanin p80 (WD repeat containing) subunit B 1	KATNB1	16q13	cytokinesis /// cell mobility /// microtubule depolymerization /// cell cycle /// mitosis
220774_at	1.737	3,33341283	dymeclin	DYM	18q12-q21.1	---
206619_at	1.406	2,65001403	dickkopf homolog 4 (Xenopus laevis)	DKK4	8p11.2-p11.1	development /// Wnt receptor signaling pathway /// negative regulation of Wnt receptor signaling pathway
207744_at	2.127	4,36808218	---	---	---	---
220111_s_at	1.243	2,38690204	transmembrane protein 16B	TMEM16B	12p13.3	---
221662_s_at	1.066	2,09362056	solute carrier family 22 (organic anion transporter), member 7	SLC22A7	6p21.2-p21.1	ion transport /// sodium ion transport /// organic anion transport
208436_s_at	1.016	2,02230416	interferon regulatory factor 7	IRF7	11p15.5	negative regulation of transcription from RNA polymerase II promoter /// transcription /// regulation of transcription, DNA-dependent /// transcription initiation from RNA polymerase II promoter /// inflammatory response /// response to DNA damage stimulu
204939_s_at	1.478	2,78562296	phospholamban	PLN	6q22.1	calcium ion transport /// muscle contraction /// circulation
207539_s_at	1.567	2,9628796	interleukin 4	IL4	5q31.1	chemotaxis /// cellular defense response /// cholesterol metabolism /// cell proliferation /// B-cell differentiation /// T-helper 2 type immune response /// connective tissue growth factor biosynthesis /// regulation of isotype switching
216291_at	1.311	2,4811346	---	---	---	---
211898_s_at	1.059	2,08348688	EPH receptor B1	EPHB1	3q21-q23	protein amino acid phosphorylation /// signal transduction /// transmembrane receptor protein tyrosine kinase signaling pathway /// neurogenesis
221062_at	1.112	2,1614508	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	HS3ST3B1	17p12-p11.2	heparan sulfate proteoglycan biosynthesis, enzymatic modification
201452_at	1.125	2,18101547	Ras homolog enriched in brain	RHEB	7q36	signal transduction /// small GTPase mediated signal transduction
216690_at	2.058	4,16408639	olfactory receptor, family 7, subfamily C, member 1	OR7C1	19p13.1	---
204471_at	1.514	2,85600798	growth associated protein 43	GAP43	3q13.1-q13.2	regulation of cell growth /// protein kinase C activation /// neurogenesis /// response to wounding /// cell differentiation
220638_s_at	1.292	2,44867278	Cas-Br-M (murine) ecotropic retroviral transforming sequence c	CBLC	19q13.2	---
206709_x_at	1.374	2,59188193	glutamic-pyruvate transaminase (alanine aminotransferase)	GPT	8q24.3	gluconeogenesis /// nitrogen compound metabolism /// biosynthesis
219082_at	1.875	3,86801617	CGI-14 protein	CGI-14	16p13.3	N-acetylglucosamine metabolism
204444_at	1.622	3,07801444	kinesin family member 11	KIF11	10q24.1	cytokinesis /// microtubule-based movement /// cell cycle /// mitotic spindle organization and biogenesis
204765_at	1.251	2,38006339	Rho guanine nucleotide exchange factor (GEF) 5	ARHGEF5	7q33-q35	---
210572_at	1.949	3,8810681	protocadherin alpha 2	PCDHA2	5q31	cell adhesion /// homophilic cell adhesion /// neurogenesis
218719_s_at	2.282	4,86351713	hypothetical protein FLJ13912	FLJ13912	16q21	---
210562_at	1.629	3,09298535	GREB1 protein	GREB1	2p25.1	---
205151_s_at	2.111	4,31990624	KIAA0644 gene product	KIAA0644	7p15.1	---
222258_s_at	1.852	3,61000291	SH3-domain binding protein 4	SH3BP4	2q37.1-q37.2	endocytosis /// cell cycle
217123_x_at	2.144	4,41985794	pro-melanin-concentrating hormone-like 1	PMCHL1	5p14.3	synaptic transmission /// behavior
204433_s_at	1.553	2,93426669	spermatogenesis associated 2	SPATA2	20q13.1-q13.2	spermatogenesis /// cell differentiation
214981_at	3.158	8,91354921	Periostin, osteoblast specific factor	POSTN	13q13.3	skeletal development /// cell adhesion /// cell adhesion
221863_at	1.07	2,09943337	KIAA1193	KIAA1193	19p13.3	---
207658_s_at	1.12	2,17346973	forkhead box G1B /// forkhead box G1A	FOXG1B /// FOXG1A	14q12-q13 /// 14q13	transcription /// regulation of transcription, DNA-dependent /// regulation of transcription, DNA-dependent /// development /// brain development /// brain development
207655_s_at	1.118	2,17045874	B-cell linker	BLNK	10q23.2-q23.33	inflammatory response /// humoral immune response /// intracellular signaling cascade /// intracellular signaling cascade /// hemocyte development /// B-cell differentiation

211812_s_at	1.023	2,03214029	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 3	B3GALT3	3q25	protein amino acid glycosylation
216155_at	2.088	4,2515827	Neuron navigator 1	NAV1	---	DNA methylation
218801_at	2.071	4,20177818	UDP-glucose ceramide glucosyltransferase-like 2	UGCGL2	13q32.1	protein amino acid glycosylation /// posttranslational protein folding
203753_at	1.511	2,85007523	transcription factor 4	TCF4	18q21.1	transcription /// regulation of transcription from RNA polymerase II promoter
207484_s_at	1.099	2,14206165	HLA-B associated transcript 8	BAT8	6p21.31	chromatin modification
215406_at	2.238	4,71742637	Triple functional domain (PTPRF interacting)	TRIO	5p15.1-p14	protein amino acid phosphorylation /// transmembrane receptor protein tyrosine phosphatase signaling pathway
211663_x_at	1.528	2,88385774	prostaglandin D2 synthase 21kDa (brain) /// prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep
200878_at	2.002	4,00554902	endothelial PAS domain protein 1	EPAS1	2p21-p16	angiogenesis /// transcription /// regulation of transcription, DNA-dependent /// transcription from RNA polymerase II promoter /// signal transduction /// cell differentiation
203443_at	1.923	3,79210786	hypothetical protein FLJ35827	FLJ35827	11q12.3	---
219463_at	2.197	4,58524874	chromosome 20 open reading frame 103	C20orf103	20p12	---
209989_at	2.376	5,19095507	zinc finger protein 268	ZNF268	12q24.33	transcription /// regulation of transcription, DNA-dependent
214418_at	2.481	5,58284306	hypothetical protein LOC196993	LOC196993	15q22.32	---
210928_at	2.131	4,38020988	CCR4-NOT transcription complex, subunit 2	CNOT2	12q15	regulation of transcription, DNA-dependent /// regulation of global transcription from RNA polymerase II promoter
218856_at	2.045	4,12673272	tumor necrosis factor receptor superfamily, member 21	TNFRSF21	6p21.1-12.2	apoptosis /// signal transduction
219832_s_at	1.75	3,36358566	homeo box C13	HOXC13	12q13.3	regulation of transcription, DNA-dependent /// morphogenesis
216325_x_at	1.054	2,07627854	regulator of telomere elongation helicase 1	RTEL1	20q13.3	nucleobase, nucleoside, nucleotide and nucleic acid metabolism /// nucleotide-excision repair
219534_x_at	2.99	7,94473998	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
216623_x_at	1.254	2,38501774	trinucleotide repeat containing 9	TNRC9	16q12.1	regulation of transcription, DNA-dependent
213568_at	1.385	2,61171957	odd-skipped related 2 (Drosophila)	OSR2	8q22.2	---
201655_s_at	1.57	2,98904714	heparan sulfate proteoglycan 2 (perlecan)	HSPG2	1p36.1-p34	cell adhesion
211748_x_at	3.055	8,31087283	prostaglandin D2 synthase 21kDa (brain) /// prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep
201820_at	1.984	3,95588367	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)	KRT5	12q12-q13	epidermis development
216894_x_at	2.89	7,4127045	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	CDKN1C	11p15.5	regulation of cyclin dependent protein kinase activity /// G1 phase of mitotic cell cycle /// cell cycle /// cell cycle arrest /// negative regulation of cell proliferation /// negative regulation of cell cycle
215047_at	1.086	2,12284642	tripartite motif-containing 58	TRIM58	1q44	protein ubiquitination
207118_s_at	1.843	3,58755284	matrix metalloproteinase 23B /// matrix metalloproteinase 23A	MMP23B /// MMP23A	1p36.3	reproduction /// proteolysis and peptidolysis /// proteolysis and peptidolysis
220837_at	2.411	5,31842843	---	---	---	---
215613_at	1.422	2,8795672	A disintegrin and metalloproteinase domain 12 (meltrin alpha)	ADAM12	10q26.3	proteolysis and peptidolysis /// cell adhesion /// myoblast fusion
207989_at	2.129	4,37414183	---	---	---	---
204817_at	1.629	3,09298535	extra spindle poles like 1 (S. cerevisiae)	ESPL1	12q	mitotic sister chromatid segregation /// regulation of cell cycle /// cytokinesis /// proteolysis and peptidolysis /// apoptosis /// chromosome segregation /// establishment of mitotic spindle localization /// positive regulation of mitotic metaphase/anap
210313_at	3.995	15,9446442	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 4	ILT7	19q13.4	immune response
206773_at	1.248	2,371829	lymphocyte antigen 6 complex, locus H	LY6H	8q24.3	cellular defense response /// neurogenesis
212187_x_at	3.211	9,25992174	prostaglandin D2 synthase 21kDa (brain)	PTGDS	9q34.2-q34.3	prostaglandin biosynthesis /// fatty acid biosynthesis /// transport /// regulation of circadian sleep/wake cycle, sleep
213997_at	2.287	4,88040204	KIAA0574 protein	KIAA0574	15q12	---
221377_s_at	1.9	3,73213197	recombining binding protein suppressor of hairless (Drosophila)-like	RBPSUHL	20q12-q13.1	transcription /// regulation of transcription, DNA-dependent /// signal transduction

Tab. A4f:
Probe-sets up-regulated regulated upon IVMP treatment at day 21 compared to day 6;
Probe-sets have a minimum change of 2-fold in 100% of patients;

Tab. A5

Probe Set ID	Gene Symbol	Gene Title	Chromosomal Location	GO Biological Process
200629_at	WARS	tryptophanyl-tRNA synthetase	14q32.31	protein biosynthesis, tryptophanyl-tRNA aminoacylation, negative regulation of cell proliferation
200678_x_at	GRN	granulin	17q21.32	signal transduction, cell-cell signaling, cell proliferation, positive regulation of cell proliferation
200986_at	SERPING1	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), mem	11q12-q13.1	immune response, complement activation, classical pathway, blood coagulation, circulation
201041_s_at	DUSP1	dual specificity phosphatase 1	5q34	protein amino acid dephosphorylation, response to oxidative stress, cell cycle
201218_at	---	---	---	
201360_at	CST3	cystatin C (amyloid angiopathy and cerebral hemorrhage)	20p11.21	
201422_at	---	---	---	
201425_at	ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)	12q24.2	carbohydrate metabolism, alcohol metabolism, metabolism
201670_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	6q22.2	cell motility
201739_at	SGK	serum/glucocorticoid regulated kinase	6q23	protein amino acid phosphorylation, sodium ion transport, apoptosis, response to stress
201743_at	CD14	CD14 antigen	5q22-q32|5q31.1	phagocytosis, apoptosis, inflammatory response, cell surface receptor linked signal transduction
201798_s_at	FER1L3	fer-1-like 3, myoferlin (C. elegans)	10q24	muscle contraction, circulation
202269_x_at	GBP1	guanylate binding protein 1, interferon-inducible, 67kDa	1p22.2	immune response
202510_s_at	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	14q32	angiogenesis; cell differentiation
202626_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	8q13	protein amino acid phosphorylation, intracellular signaling cascade
202833_s_at	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	14q32.1	acute-phase response
202897_at	PTPNS1	protein tyrosine phosphatase, non-receptor type substrate 1	20p13	
203066_at	GALNAC4S-6ST	B cell RAG associated protein	10q26	regulation of DNA recombination, hexose biosynthesis, regulation of B-cell differentiation
203153_at	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	10q25-q26	immune response
203535_at	S100A9	S100 calcium binding protein A9 (calgranulin B)	1q21	inflammatory response, cell-cell signaling
203561_at	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	1q23	immune response
203922_s_at	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	Xp21.1	electron transport, ion transport, inflammatory response, antimicrobial humoral response (sensu Vertebrata)
203923_s_at	CYBB	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	Xp21.1	electron transport, ion transport, inflammatory response, antimicrobial humoral response (sensu Vertebrata)
204039_at	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	19q13.1	generation of precursor metabolites and energy, transcription, regulation of transcription, DNA-dependent, transcription from RNA polymerase II promoter
204122_at	TYROBP	TYRO protein tyrosine kinase binding protein	19q13.1	cellular defense response, intracellular signaling cascade
204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; γ-polypeptide	1q23	immune response, cell surface receptor linked signal transduction
204249_s_at	LMO2	LIM domain only 2 (rhombotin-like 1)	11p13	development
204415_at	G1P3	interferon, alpha-inducible protein (clone IFI-6-16)	1p35	immune response, response to pest, pathogen or parasite
204445_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
204446_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
204533_at	CXCL10	chemokine (C-X-C motif) ligand 10	4q21	cell motility; chemotaxis; inflammatory response; cell surface receptor linked signal transduction
204588_s_at	SLC7A7	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	14q11.2	protein complex assembly, amino acid metabolism, transport, transport, amino acid transport
204619_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
204620_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition
204747_at	IFIT3	interferon-induced protein with tetratricopeptide repeats 3	10q24	immune response
204834_at	FGL2	fibrinogen-like 2	7q11.23	
204858_s_at	ECGF1	endothelial cell growth factor 1 (platelet-derived)	22q13|22q13.33	mitochondrial genome maintenance, angiogenesis, pyrimidine base metabolism
204924_at	TLR2	toll-like receptor 2	4q32	pyrimidine nucleotide metabolism, DNA replication, chemotaxis, cell surface receptor linked signal transduction, cell-cell signaling, sensory perception, metabolism, cell differentiation
204959_at	MNDA	myeloid cell nuclear differentiation antigen	1q22	induction of apoptosis, inflammatory response, signal transduction
204961_s_at	NCF1	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease	7q11.23	transcription, regulation of transcription, DNA-dependent, cellular defense response
204971_at	CSTA	cystatin A (stefin A)	3q21	electron transport; superoxide metabolism; cellular defense response; intracellular signaling cascade
205076_s_at	MTMR11	myotubularin related protein 11	1q12-q21	phospholipid dephosphorylation
205119_s_at	FPR1	formyl peptide receptor 1	19q13.4	activation of MAPK, cell motility, chemotaxis, inflammatory response, signal transduction
205237_at	FCN1	ficolin (collagen/fibrinogen domain containing) 1	9q34	phosphate transport, cell adhesion, opsonization
205312_at	SPI1	spleen focus forming virus (SFFV) proviral integration oncogene sp	11p11.2	negative regulation of transcription from RNA polymerase II promoter, transcription, regulation of transcription, DNA-dependent
205483_s_at	G1P2	interferon, alpha-inducible protein (clone IFI-15K)	1p36.33	protein modification, immune response, cell-cell signaling
205715_at	BST1	bone marrow stromal cell antigen 1	4p15	humoral immune response, development
205789_at	CD1D	CD1D antigen, d polypeptide	1q22-q23	detection of bacteria, T-cell selection, positive regulation of innate immune response, antigen presentation, endogenous peptide antigen, antigen presentation, endogenous lipid antigen
205863_at	S100A12	S100 calcium binding protein A12 (calgranulin C)	1q21	xenobiotic metabolism, inflammatory response, defense response to bacteria, defense response to fungi
205936_s_at	HK3	hexokinase 3 (white cell)	5q35.2	glycolysis
206133_at	HSXIAPAF1	XIAP associated factor-1	17p13.1	
206380_s_at	PFC	properdin P factor, complement	Xp11.3-p11.23	immune response, complement activation, alternative pathway, defense response to bacteria
206710_s_at	EPB41L3	erythrocyte membrane protein band 4.1-like 3	18p11.32	cortical actin cytoskeleton organization and biogenesis
207078_at	MED6	mediator of RNA polymerase II transcription, subunit 6 homolog (ye	14q24.2	regulation of transcription from RNA polymerase II promoter
207104_x_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	19q13.4	immune response, response to virus
207540_s_at	SYK	spleen tyrosine kinase	9q22	protein complex assembly, protein amino acid phosphorylation, leukocyte cell adhesion, integrin-mediated, signaling pathway, intracellular signaling cascade, cell proliferation, cell proliferation, organogenesis, neutrophil chemotaxis
207610_s_at	EMR2	egf-like module containing, mucin-like, hormone receptor-like 2	19p13.1	signal transduction; G-protein coupled receptor protein signaling pathway; neuropeptide signaling pathway
207697_x_at	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	19q13.4	cellular defense response; cell surface receptor signal transduction, antimicrobial humoral response
207857_at	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	19q13.4	immune response; signal transduction
208018_s_at	HCK	hemopoietic cell kinase	20q11-q12	protein amino acid phosphorylation, intracellular signaling cascade, mesoderm development
208130_s_at	TBXAS1	thromboxane A synthase 1 (platelet, cytochrome P450, family 5, su	7q34-q35	prostaglandin biosynthesis, electron transport, fatty acid biosynthesis, transport, blood coagulation
208594_x_at	LILRB6	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 6	19q13.4	
208890_s_at	PLXNB2	plexin B2	22q13.33	development
208891_at	DUSP6	dual specificity phosphatase 6	12q22-q23	regulation of cell cycle; inactivation of MAPK protein amino acid dephosphorylation
208892_s_at	DUSP6	dual specificity phosphatase 6	12q22-q23	regulation of cell cycle; inactivation of MAPK protein amino acid dephosphorylation
209189_at	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	14q24.3	DNA methylation, regulation of transcription from RNA polymerase II promoter, inflammatory response
209500_x_at	TNFSF13	tumor necrosis factor (ligand) superfamily, member 13	17p13.1	immune response, signal transduction, positive regulation of cell proliferation
209683_at	FAM49A	Family with sequence similarity 49, member A	2p24.3-p24.2	
209684_at	RIN2	Ras and Rab interactor 2	---	endocytosis, intracellular signaling cascade, small GTPase mediated signal transduction
209906_at	C3AR1	complement component 3a receptor 1	12p13.31	cell motility, chemotaxis, smooth muscle contraction, inflammatory response, cellular defense response, signal transduction, G-protein coupled receptor protein signaling pathway, neuropeptide signaling pathway, positive regulation of cytosolic calcium ion concentration, sensory perception, circulation
209949_at	NCF2	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	1q25	superoxide metabolism, cellular defense response
209969_s_at	STAT1	signal transducer and activator of transcription 1, 91kDa	2q32.2	regulation of cell cycle transcription regulation of transcription, DNA-dependent transcription from RNA polymerase II promoter, caspase activation, intracellular signaling cascade, I-kappaB kinase/NF-kappaB cascade, tyrosine phosphorylation of STAT protein, STAT protein nuclear translocation, response to pest, pathogen or parasite
210146_x_at	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	19q13.4	cellular defense response; cell surface receptor signal transduction; antimicrobial humoral response
210148_at	HIPK3	homeodomain interacting protein kinase 3	11p13	transcription, regulation of transcription, DNA-dependent, protein amino acid phosphorylation, apoptosis
210222_s_at	RTN1	reticulon 1	14q23.1	signal transduction, neuron cell differentiation
210423_s_at	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transport	2q35	transport, iron ion transport, response to pest, pathogen or parasite, response to bacteria
210629_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis, negative regulation of lymphocyte proliferation
210660_at	LILRA1	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 1	19q13.4	immune response, cell surface receptor linked signal transduction
210663_s_at	KYNU	kynureninase (L-kynurenine hydrolase)	2q22.3	protein amino acid phosphorylation, intracellular signaling cascade
210754_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	8q13	immune response, cell-cell signaling, positive regulation of cell proliferation, T-cell activation, positive
210873_x_at	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	22q13.1-q13.2	
210895_s_at	CD86	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	3q21	immune response, cell-cell signaling, positive regulation of cell proliferation, T-cell activation, positive regulation of tumor necrosis factor-beta biosynthesis, positive regulation of interleukin-2 biosynthesis,

				positive regulation of interleukin-4 biosynthesis, positive regulation of T-helper 2 cell differentiation, positive regulation of transcription
211100_x_at	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	19q13.4	immune response; signal transduction
211101_x_at	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	19q13.4	immune response; signal transduction
211284_s_at	GRN	granulin	17q21.32	signal transduction, cell-cell signaling, cell proliferation, positive regulation of cell proliferation
211338_x_at	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	19q13.4	immune response, response to virus
211429_s_at	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	14q32.1	acute-phase response
211582_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis, negative regulation of lymphocyte proliferation
212099_at	RHOB	ras homolog gene family, member B	2p24	angiogenesis; programmed cell death; transformed cells; cell cycle; cell adhesion small GTPase mediated signal transduction; endosome to lysosome transport; protein transport cell differentiation; positive regulation of angiogenesis; negative regulation of cell cycle
212192_at	KCTD12	potassium channel tetramerisation domain containing 12	13q22.3	potassium ion transport
212225_at	SUI1	putative translation initiation factor	17q21.2	protein biosynthesis, translational initiation, regulation of translation, regulation of translational initiation, response to stress
212636_at	QKI	quaking homolog, KH domain RNA binding (mouse)	6q26-27	
212681_at	EPB41L3	erythrocyte membrane protein band 4.1-like 3	18p11.32	cortical actin cytoskeleton organization and biogenesis
213182_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle, cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
213418_at	HSPA6	heat shock 70kDa protein 6 (HSP70B')	1q23	protein folding; response to unfolded protein
213472_at	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)	5q35.3	RNA processing
213524_s_at	G0S2	putative lymphocyte G0/G1 switch gene	1q32.2-q41	regulation of cell cycle
213566_at	RNASE6	ribonuclease, RNase A family, k6	14q11.2	RNA catabolism, defense response
213716_s_at	SECTM1	secreted and transmembrane 1	17q25	immune response, mesoderm development, positive regulation of I-kappaB kinase/NF-kappaB cascade
214038_at	CCL8	chemokine (C-C motif) ligand 8	17q11.2	calcium ion transport, exocytosis, chemotaxis, inflammatory response, signal transduction, cell cycle arrest,
214084_x_at	---	---	---	
214366_s_at	ALOX5	arachidonate 5-lipoxygenase	10q11.2	electron transport, inflammatory response, leukotriene biosynthesis
214511_x_at	---	---	---	
214722_at	NOTCH2NL	Notch homolog 2 (Drosophila) N-terminal like	1q21.2	
215123_at	---	---	---	
215220_s_at	TPR	translocated promoter region (to activated MET oncogene)	1q25	protein-nucleus import, transport
215633_x_at	LST1	leukocyte specific transcript 1	6p21.3	cellular morphogenesis, immune response, immune response, dendrite morphogenesis, negative regulation of lymphocyte proliferation
215933_s_at	HHEX	hematopoietically expressed homeobox	10q23.33	regulation of transcription, DNA-dependent, development, antimicrobial humoral response
216041_x_at	GRN	granulin	17q21.32	signal transduction, cell-cell signaling, cell proliferation, positive regulation of cell proliferation
216109_at	THRAP2	Thyroid hormone receptor associated protein 2	12q24.21	
216894_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle, cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
216899_s_at	SCAP2	src family associated phosphoprotein 2	7p21-p15	protein complex assembly, signal transduction
216950_s_at	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	1q21.2-q21.3	phagocytosis, engulfment; immune response
217388_s_at	KYNU	kynureninase (L-kynurenine hydrolase)	2q22.3	tryptophan catabolism, NAD biosynthesis
217679_x_at	---	---	---	
217763_s_at	RAB31	RAB31, member RAS oncogene family	18p11.3	small GTPase mediated signal transduction
217764_s_at	RAB31	RAB31, member RAS oncogene family	18p11.3	small GTPase mediated signal transduction
217853_at	TENS1	tensin-like SH2 domain containing 1	7p13-p12.3	protein amino acid dephosphorylation, cell cycle, intracellular signaling cascade
218035_s_at	FLJ20273	RNA-binding protein	4p13-p12	
218559_s_at	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B	20q11.2-q13.1	transcription, regulation of transcription, DNA-dependent, sensory organ development
219093_at	FLJ20701	hypothetical protein FLJ20701	2q36.3	
219519_s_at	SN	sialoadhesin	20p13	inflammatory response, cell-matrix adhesion, cell-cell adhesion
219534_x_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	11p15.5	regulation of cyclin dependent protein kinase activity, G1 phase of mitotic cell cycle, cell cycle, cell cycle arrest, negative regulation of cell proliferation, negative regulation of cell cycle
219607_s_at	MS4A4A	membrane-spanning 4-domains, subfamily A, member 4	11q12	signal transduction
219788_at	PILRA	paired immunoglobin-like type 2 receptor alpha	7q22.1	transmembrane receptor protein tyrosine kinase, activation (dimerization)
220088_at	C5R1	complement component 5 receptor 1 (C5a ligand)	19q13.3-q13.4	activation of MAPK;chemotaxis; G-protein coupled receptor protein signaling pathway phospholipase C activation; positive regulation of cytosolic calcium ion concentration sensory perception of chemical stimulus
220091_at	SLC2A6	solute carrier family2 (facilitated glucose transporter), member 6	9q34	carbohydrate transport
220146_at	---	---	---	
220532_s_at	LR8	LR8 protein	7q36.1	organogenesis
221060_s_at	TLR4	toll-like receptor 4	9q32-q33	inflammatory response, signal transduction, activation of NF-kappaB-inducing kinase, detection of pathogenic bacteria, detection of fungi, T-helper 1 type immune response, macrophage activation, positive regulation of interleukin-12 biosynthesis, positive regulation of interleukin-1 biosynthesis, positive regulation of interleukin-13 biosynthesis, positive regulation of interleukin-6 biosynthesis mast cell activation, negative regulation of osteoclast differentiation
221581_s_at	WBSCR5	Williams-Beuren syndrome chromosome region 5	7q11.23	intracellular signaling cascade, calcium-mediated signaling, B-cell activation
221698_s_at	CLEC7A	C-type lectin domain family 7, member A	12p13.2-p12.3	phagocytosis, recognition, cell recognition, carbohydrate mediated signaling, antibacterial humoral response (sensu Vertebrata), antifungal humoral response (sensu Vertebrata), T-cell activation, defense response to pathogenic protozoa, reduction of virulence
221731_x_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	5q14.3	development, cell recognition

Tab. A5:
Subtraction of IVMP-specific genes after treatment of IVIG-specific genes after treatment;
Probe-sets listed above are specifically differentially regulated upon IVIG therapy;
Criteria for IVIG treatment: a minimum of a 2-fold change in at least 40% of patients (4 out of 10);
Criteria for IVMP treatment: a minimum of a 2-fold change in at least 60% of patients (3 out of 5);

Tab. A6				
Probe Set ID	Gene Title	Gene Symbol	Chromosomal Location	GO Biological Process Description
204624_at	AFP	alpha-fetoprotein	4q11-q13	transport, immune response
204895_s_at	PTGER4	prostaglandin E receptor 4 (subtype EP4)	5p13.1	immune response, signal transduction, G-protein coupled receptor protein signaling pathway,
				G-protein signaling, coupled to cAMP nucleotide second messenger
204936_at	MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2	11q13	protein amino acid phosphorylation, vesicle targeting, response to stress, immune response,
				protein kinase cascade, JNK cascade, hemocyte development
206366_x_at	XCL2	chemokine (C motif) ligand 2	1q23-q25	chemotaxis, immune response, signal transduction, cell-cell signaling, sensory perception, circulation
206407_s_at	CCL13	chemokine (C-C motif) ligand 13	17q11.2	calcium ion homeostasis, chemotaxis, inflammatory response, signal transduction, cell-cell signaling,
				sensory perception
208198_x_at	KIR2DS1	killer cell immunoglobulin-like receptor,	19q13.4	immune response
		two domains, short cytoplasmic tail, 1		
207850_at	CXCL3	chemokine (C-X-C motif) ligand 3	4q21	chemotaxis, inflammatory response, G-protein coupled receptor protein signaling pathway,
				sensory perception
207852_at	CXCL5	chemokine (C-X-C motif) ligand 5	4q12-q13	chemotaxis, inflammatory response, signal transduction, cell-cell signaling,
				positive regulation of cell proliferation
219859_at	CLEC4E	C-type lectin domain family 4, member E	12p13.31	immune response, antimicrobial humoral response (sensu Vertebrata)
204531_s_at	BRCA1	breast cancer 1, early onset	17q21	cell cycle checkpoint, DNA repair, regulation of transcription from RNA polymerase II promoter,
				regulation of transcription from RNA polymerase III promoter, DNA damage response,
				signal transduction by p53, class mediator resulting in transcription of p21 class mediator, cell cycle,
				protein ubiquitination, regulation of cell proliferation, regulation of apoptosis,regulation of cell positive
				regulation of DNA repair, negative regulation of cell cycle , negative regulation of centriole replication
204406_at	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth	13q12	angiogenesis, protein amino acid phosphorylation, transmembrane receptor protein tyrosine kinase
		factor/vascular permeability factor receptor)		signaling pathway, pregnancy, positive regulation of cell proliferation, cell differentiation
204887_s_at	PLK4	polo-like kinase 4 (Drosophila)	4q27-q28	regulation of cell cycle, protein amino acid phosphorylation
206754_at	F2	coagulation factor II (thrombin)	11p11-q12	regulation of cell cycle, proteolysis and peptidolysis, apoptosis, caspase activation, acute-phase ,
				response, tyrosine phosphorylation of STAT protein, STAT protein nuclear translocation, development
				response to wounding, platelet activation, regulation of blood coagulation, fibrinolysis
206926_s_at	IL11	interleukin 11	19q13.3-q13.4	cell-cell signaling, positive regulation of cell proliferation, platelet activation, B-cell differentiation,
				megakaryocyte differentiation, adipocyte differentiation
209485_x_at	PTN	pleiotrophin (heparin binding growth factor 8,	7q33-q34	regulation of cell cycle, transmembrane receptor protein tyrosine phosphatase signaling pathway,
		neurite growth-promoting factor 1)		neurogenesis, cell proliferation, positive regulation of cell proliferation
210358_at	MTSS1	metastasis suppressor 1	8p22	cell motility, cell cycle, cell adhesion, transmembrane receptor protein tyrosine kinase signaling
				pathway, neurogenesis, muscle development , microspike biogenesis, actin cytoskeleton organization
				and biogenesis, negative regulation of cell cycle
210960_at	ADRA1D	adrenergic, alpha-1D-, receptor	20p13	DNA metabolism, signal transduction, G-protein coupled receptor protein signaling pathway,
				G-protein signaling, coupled to cAMP nucleotide second messenger,
				cell-cell signaling, development, cell proliferation, positive regulation of cell proliferation
211154_at	THPO	thrombopoietin (myeloproliferative leukemia virus oncogene	3q27	development, cell proliferation
		ligand, megakaryocyte growth and development factor)		
211792_s_at	CDKN2C	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	1p32	cell cycle, cell cycle arrest, negative regulation of cell proliferation
211831_s_at	THPO	thrombopoietin (myeloproliferative leukemia virus oncogene	3q27	development, cell proliferation
		ligand,megakaryocyte growth and development factor)		
214381_at	LOC441601	similar to CDC10 (cell division cycle 10, S.cerevisiae, homolog)	11p11.12	cell cycle
215070_x_at	RABGAP1	RAB GTPase activating protein 1	9q33.2-q33.3	cell cycle
215381_at	FRAP1	FK506 binding protein 12-rapamycin associated protein 1	1p36.2	regulation of cell cycle, DNA repair, DNA recombination
215824_at	TSC2	Tuberous sclerosis 2	16p13.3	protein folding, endocytosis, negative regulation of cell cycle
215793_at	MTMR7	myotubularin related protein 7	8p22	protein amino acid dephosphorylation, protein amino acid dephosphorylation, cell cycle,
				phospholipid dephosphorylation
215942_s_at	GTSE1	G-2 and S-phase expressed 1	22q13.2-q13.3	G2 phase of mitotic cell cycle, DNA damage response, signal transduction by p53 class mediator
				resulting in cell cycle arrest, microtubule-based process
216896_at	COL4A3	collagen, type IV, alpha 3 (Goodpasture antigen)	2q36-q37	proteolysis and peptidolysis, phosphate transport, induction of apoptosis, caspase activation,
				cell adhesion, cell surface receptor linked signal transduction, perception of sound, circulation,
				cell proliferation, negative regulation of cell proliferation and angiogenesis
222305_at	HK2	hexokinase 2	2p13	regulation of cell cycle, glycolysis
204833_at	ARG12	ARG12 (ARG1 [illegible] (S. cerevisiae)	5q21-q22	[illegible]
204640_at	CASP2	caspase 2 (apoptosis-related cysteine protease) (neural	7q34-q35	proteolysis and peptidolysis, proteolysis and peptidolysis, induction of apoptosis, apoptosis,
		precursor cell expressed developmentally down-regulated 2)		regulation of apoptosis
201042_at	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glut	20q12	G-protein coupled receptor protein signaling pathway, peptide cross-linking,
				positive regulation of cell adhesion
201124_at	ITGB5	integrin, beta 5	3q21.2	cell-matrix adhesion, integrin-mediated signaling pathway, development
201430_s_at	DPYSL3	dihydropyrimidinase-like 3	5q32	nucleobase, nucleoside, nucleotide and nucleic acid metabolism, signal transduction, neurogenesis
203499_at	EPHA2	EPH receptor A2	1p36	protein amino acid phosphorylation, signal transduction, transmembrane receptor protein tyrosine
				kinase signaling pathway, development
204591_at	CHL1	cell adhesion molecule with homology to L1CAM	3p26.1	cell adhesion, signal transduction
205056_s_at	GRCA	gene rich cluster, A gene	12p13	G-protein coupled receptor protein signaling pathway, protein metabolism
206223_at	DEPDC5	DEP domain containing 5	22q12.3	intracellular signaling cascade
206552_s_at	TAC1	tachykinin, precursor 1 (substance K, substance P, neurokinin 1, n	7q21-q22	tachykinin signaling pathway, neuropeptide signaling pathway, cell-cell signaling, synaptic
				transmission, insemination, detection of abiotic stimulus
206803_at	PDYN	prodynorphin	20pter-p12	neuropeptide signaling pathway, synaptic transmission
207333_at	NMBR	neuromedin B receptor	6q21-qter	signal transduction, G-protein signaling, coupled to IP3 second messenger (phospholipase C activating)
207557_s_at	RYR2	ryanodine receptor 2 (cardiac)	1q42.1-q43	cation transport, calcium ion transport, calcium ion homeostasis, muscle contraction,
				signal transduction, regulation of heart contraction rate
206222_at	ACVR1B	activin A receptor, type IB	12q13	protein amino acid phosphorylation, transmembrane receptor protein
				serine/threonine kinase signaling pathway
209886_s_at	LPHN3	latrophilin 3	4q13.1	signal transduction, neuropeptide signaling pathway
210880_s_at	EFS	embryonal Fyn-associated substrate	14q11.2-q12	cell adhesion, intracellular signaling cascade
211448_s_at	RGS6	regulator of G-protein signalling 6	14q24.3	G-protein coupled receptor protein signaling pathway, intracellular signaling cascade,
				regulation of G-protein coupled receptor protein signaling pathway
213516_at	AKAP13	A kinase (PRKA) anchor protein 13	15q24-q25	intracellular signaling cascade
214361_s_at	RGS12	regulator of G-protein signalling 12	4p16.3	signal transduction, regulation of G-protein coupled receptor protein signaling pathway, protein transport
215618_at	RSU1	Ras suppressor protein 1	10p13	signal transduction
215830_at	SHANK2	SH3 and multiple ankyrin repeat domains 2	11q13.3-q13.4	intracellular signaling cascade
216206_x_at	MAP2K7	mitogen-activated protein kinase kinase 7	19p13.3-p13.2	protein amino acid phosphorylation, response to stress, signal transduction
216834_at	PLCL3	phospholipase C-like 3	3q25.31	lipid metabolism, intracellular signaling cascade
221922_at	GPSM2	G-protein signaling modulator 2 (AGS3-like, C. elegans)	1p13.3	signal transduction, G-protein coupled receptor protein signaling pathway
205249_at	EGR2	early growth response 2 (Krox-20 homolog, Drosophila)	10q21.1	transcription, regulation of transcription, DNA-dependent, brain development,
				peripheral nervous system development, mechanosensory behavior
205517_at	GATA4	GATA binding protein 4	8p23.1-p22	transcription, regulation of transcription, DNA-dependent, transcription from RNA polymerase II
				promoter, development, heart development , positive regulation of transcription, DNA-dependent
205906_at	FOXJ1	forkhead box J1	17q22-17q25	transcription, regulation of transcription, DNA-dependent, spermatogenesis
206104_at	ISL1	ISL1 transcription factor, LIM/homeodomain, (islet-1)	5q11.2	regulation of transcription, DNA-dependent, development
207662_at	TBX1	T-box 1	22q11.21	transcription, regulation of transcription from RNA polymerase II promoter,
				heart development, morphogenesis
208033_s_at	ATBF1	AT-binding transcription factor 1	16q22.3-q23.1	regulation of transcription, DNA-dependent, transcription from RNA polymerase II promoter
211223_at	PROP1	prophet of Pit1, paired-like homeodomain transcription factor	5q35.3	regulation of transcription, DNA-dependent, central nervous system development
211627_x_at	ESR1	estrogen receptor 1	6q25.1	regulation of transcription, DNA-dependent, regulation of transcription, DNA-dependent, signal
				transduction, cell growth, estrogen receptor signaling pathway, negative regulation of mitosis
212258_s_at	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of	9p22.3	transcription, DNA-dependent, regulation of transcription from RNA polymerase II promoter, cell cycle
214451_at	TFAP2B	transcription factor AP-2 beta	6p21-p12	transcription, regulation of transcription from RNA polymerase II promoter, neurogenesis
		(activating enhancer binding protein 2 beta)		
214360_at	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of	19p13.2	transcription, regulation of transcription from RNA polymerase II promoter
47550_at	LZTS1	leucine zipper, putative tumor suppressor 1	8p22	transcription, regulation of transcription, DNA-dependent, cell cycle, negative regulation of cell cycle

201616_s_at	CALD1	caldesmon 1	7q33	muscle contraction, muscle development
201961_s_at	RNF41	ring finger protein 41	12q13.2-q13.3	protein ubiquitination
202563_at	C14orf1	chromosome 14 open reading frame 1	14q24.3	sterol biosynthesis
203431_s_at	RICS	Rho GTPase-activating protein	11q24-q25	---
203702_s_at	TTLL4	tubulin tyrosine ligase-like family, member 4	2p24.3-p24.1	protein modification
203759_at	ST3GAL4	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	11q23-q24	protein amino acid glycosylation
203806_s_at	FANCA	Fanconi anemia, complementation group A	16q24.3	DNA repair, protein complex assembly
204056_s_at	MVK	mevalonate kinase (mevalonic aciduria)	12q24	protein amino acid phosphorylation, cholesterol biosynthesis, isoprenoid biosynthesis
204257_at	FADS3	fatty acid desaturase 3	11q12-q13.1	fatty acid biosynthesis, fatty acid desaturation, fatty acid desaturation
204414_at	---	---	---	---
204482_at	CLDN5	claudin 5 (transmembrane protein deleted	22q11.21	calcium-independent cell-cell adhesion
		in velocardiofacial syndrome)		
204643_s_at	COVA1	cytosolic ovarian carcinoma antigen 1	Xq25-q26.2	regulation of cell growth, electron transport, transport, ultradian rhythm
204811_s_at	CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	3p21.3	ion transport, calcium ion transport
204816_s_at	DHX34	DEAH (Asp-Glu-Ala-His) box polypeptide 34	19q13.3	electron transport
205024_s_at	RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)	15q15.1	double-strand break repair via homologous recombination, double-strand break repair via homologous
				recombination, DNA unwinding, DNA unwinding, DNA repair, mitotic recombination, meiosis,
				meiotic recombination, positive regulation of DNA ligation, protein homooligomerization
205028_at	TRO	trophinin	Xp11.22-p11.21	cell adhesion, homophilic cell adhesion, embryo implantation
205072_s_at	XRCC4	X-ray repair complementing defective repair in	5q13-q14	DNA repair, double-strand break repair, DNA recombination
		Chinese hamster cells 4		
205122_at	TMEFF1	transmembrane protein with EGF-like and	9q31	---
		two follistatin-like domains 1		
205317_s_at	SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	3q13.33	transport, oligopeptide transport
205360_at	PFDN4	prefoldin 4	20q13.2	protein folding, chaperonin-mediated tubulin folding
205498_at	GHR	growth hormone receptor	5p13-p12	skeletal development, endocytosis, growth
205649_s_at	FGA	fibrinogen, A alpha polypeptide	4q28	blood coagulation, regulation of blood pressure, positive regulation of cell proliferation
205962_at	PAK2	p21 (CDKN1A)-activated kinase 2	3q29	protein amino acid phosphorylation, protein amino acid phosphorylation, negative regulation
				of protein kinase activity, signal transduction
206043_s_at	KIAA0703	KIAA0703 gene product	16q24.1	cation transport, calcium ion transport, metabolism, proton transport
206265_s_at	GPLD1	glycosylphosphatidylinositol specific phospholipase D1	6p22.3-p22.2	cell-matrix adhesion
206294_at	HSD3B2	hydroxy-delta-5-steroid dehydrogenase,	1p13.1	C21-steroid hormone biosynthesis
		3 beta- and steroid delta-isomerase 2		
206344_at	PON1	paraoxonase 1	7q21.3	response to external stimulus
206414_s_at	DDEF2	development and differentiation enhancing factor 2	2p25|2p24	regulation of GTPase activity
206423_at	ANGPTL7	angiopoietin-like 7	1p36.3-p36.2	response to oxidative stress
206446_s_at	ELA2A	elastase 2A	1p36.21	proteolysis and peptidolysis
206519_x_at	---	---	---	---
206595_at	CST6	cystatin E/M	11q13	morphogenesis
206609_at	MAGEC1	melanoma antigen family C, 1	Xq26	---
206863_x_at	---	---	---	---
206903_at	---	---	---	---
206951_at	HIST1H4E	histone 1, H4e	6p21.3	---
207174_at	GPC5	glypican 5	13q32	---
207201_s_at	SLC22A1	solute carrier family 22 (organic cation transporter), member 1	6q26	ion transport, sodium ion transport, organic cation transport
207228_at	PRKACG	protein kinase, cAMP-dependent, catalytic, gamma	9q13	protein amino acid phosphorylation, spermatogenesis, male gonad development
207462_at	GLRA2	glycine receptor, alpha 2	Xp22.1-p21.3	ion transport, chloride transport, cell surface receptor linked signal transduction, synaptic transmission
207567_at	SLC13A2	solute carrier family 13 (sodium-dependent dicarboxylate	17p13.2	ion transport, sodium ion transport
		transporter), member 2		
207834_at	FBLN1	fibulin 1	22q13.31	chitin metabolism, development
207836_s_at	RBPMS	RNA binding protein with multiple splicing	8p12-p11	RNA processing
208153_s_at	FAT2	FAT tumor suppressor homolog 2 (Drosophila)	5q32-q33	cell adhesion, homophilic cell adhesion
208291_s_at	TH	tyrosine hydroxylase	11p15.5	synaptic transmission, aromatic amino acid family metabolism, morphogenesis,
				neurotransmitter biosynthesis, catecholamine biosynthesis
208324_at	---	---	---	---
208514_at	KCNE1	potassium voltage-gated channel, Isk-related family, member 1	21q22.1-q22.2	ion transport, potassium ion transport, muscle contraction, perception of sound,
				regulation of heart contraction rate
208580_x_at	HIST1H4K/J	histone 1, H4k/j	6p22-p21.3	---
208906_at	BSCL2	Bernardinelli-Seip congenital lipodystrophy 2 (seipin)	11q12-q13.5,11q12.3	---
209169_at	GPM6B	glycoprotein M6B	Xp22.2	neurogenesis, cell differentiation
209299_x_at	PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2	22q11.21	protein folding
209346_s_at	PI4KII	phosphatidylinositol 4-kinase type II	10q24	phosphatidylinositol biosynthesis
209437_s_at	SPON1	spondin 1, extracellular matrix protein	11p15.2	cell adhesion, development
209590_at	BMP7	Bone morphogenetic protein 7 (osteogenic protein 1)	20q13	skeletal development, cell differentiation, growth
209612_s_at	ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	4q21-q23	ethanol oxidation
209615_s_at	PAK1	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	11q13-q14	protein amino acid phosphorylation, protein amino acid phosphorylation, apoptosis, JNK cascade
209676_at	TFPI	tissue factor pathway inhibitor	2q31-q32.1	blood coagulation
		(lipoprotein-associated coagulation inhibitor)		
210019_at	CALML3	calmodulin-like 3	10pter-p13	---
210066_s_at	AQP4	aquaporin 4	18q11.2-q12.1	transport, neurogenesis, excretion
210364_at	SCN2B	sodium channel, voltage-gated, type II, beta	11q23	ion transport, sodium ion transport, synaptic transmission
210429_at	RHD	Rhesus blood group, D antigen	1p36.11	---
210553_x_at	PCSK6	proprotein convertase subtilisin/kexin type 6	15q26.3	proteolysis and peptidolysis, cell-cell signaling
210702_s_at	PTGIS	prostaglandin I2 (prostacyclin) synthase	20q13.13	prostaglandin biosynthesis, electron transport, lipid metabolism, fatty acid biosynthesis
210795_s_at	MEG3	Maternally expressed 3	14q32	---
210801_at	HSA9761	dimethyladenosine transferase	5q11-q14	rRNA modification, rRNA processing
210810_s_at	SLC6A5	solute carrier family 6 (neurotransmitter transporter, glycine),	11p15.2-p15.1	neurotransmitter transport, synaptic transmission
		member 5		
211224_s_at	ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11	2q24	transport
211238_at	ADAM7	a disintegrin and metalloproteinase domain 7	8p21.2	proteolysis and peptidolysis
211259_s_at	BMP7	bone morphogenetic protein 7 (osteogenic protein 1)	20q13	skeletal development, cell differentiation, growth
211334_at	MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	11q21	regulation of mitotic recombination, double-strand break repair via nonhomologous end-joining,
				telomerase-dependent telomere maintenance , meiosis, meiotic recombination
211353_at	LRRC21	leucine rich repeat containing 21	10q23	---
211480_s_at	SLCO1A2	solute carrier organic anion transporter family, member 1A2	12p12	transport, ion transport, organic anion transport
212396_s_at	KIAA0090	KIAA0090	1p36.13	---
212478_at	FLJ13910	hypothetical protein FLJ13910	2p11.2	---
213258_at	TFPI	Tissue factor pathway inhibitor	2q31-q32.1	blood coagulation
		(lipoprotein-associated coagulation inhibitor)		
213458_at	KIAA0974	KIAA0974	10q22.2	---
213712_at	ELOVL2	elongation of very long chain fatty acids	6p24.1	fatty acid biosynthesis
		(FEN1/Elo2, SUR4/Elo3, yeast)-like 2		
213862_at	PNPLA2	Patatin-like phospholipase domain containing 2	11p15.5	---
213904_at	---	Clone 23555 mRNA sequence	---	---
213999_at	MGC11061	Hypothetical protein MGC11061	2p22.3	---
214019_at	---	---	---	---
214095_at	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	12q12-q14	glycine metabolism, L-serine metabolism, one-carbon compound metabolism
214099_s_at	PDE4DIP	phosphodiesterase 4D interacting protein (myomegalin)	1q12	protein biosynthesis, (actin) cytoskeleton organization and biogenesis
214123_s_at	C4orf10	chromosome 4 open reading frame 10	4p16.3	---
214126_at	MCART1	Mitochondrial carrier triple repeat 1	9p13.3-p12	transport
214133_at	MUC6	mucin 6, gastric	11p15.5-p15.4	---
214160_at	---	---	---	---
214507_s_at	EXOSC2	exosome component 2	9q34	rRNA processing
214530_x_at	EPB41	erythrocyte membrane protein band 4.1	1p33-p32	circulation, cortical actin cytoskeleton organization and biogenesis
		(elliptocytosis 1, RH-linked)		
214579_at	DJ462O23.2	hypothetical protein dJ462O23.2	1p36.12-p35.1	---
214588_s_at	MFAP3	Microfibrillar-associated protein 3	5q32-q33.2	---
214590_s_at	UBE2D1	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	10q11.2-q21	ubiquitin-dependent protein catabolism, ubiquitin cycle
214676_x_at	MUC3B	mucin 3B	7q22	---
214689_at	PAPPA2	pappalysin 2	1q23-q25	regulation of cell growth, regulation of cell growth, proteolysis and peptidolysis, cell differentiation
214692_s_at	JRK	jerky homolog (mouse)	8q24.3	---
214708_at	SNTB1	syntrophin, beta 1	8q23-q24	muscle contraction
		(dystrophin-associated protein A1, 59kDa, basic component 1)		
214716_at	BMP2K	BMP2 inducible kinase	4q21.21	protein amino acid phosphorylation
214803_at	---	CDNA clone IMAGE:4152983, partial cds	---	---

214929_s_at	KIAA1109	KIAA1109	4q27	---
215013_s_at	USP34	ubiquitin specific protease 34	2p15	ubiquitin-dependent protein catabolism, ubiquitin cycle
215076_s_at	COL3A1	collagen, type III, alpha 1	2q31	phosphate transport, circulation, organogenesis
		(Ehlers-Danlos syndrome type IV, autosomal dominant)		
215200_x_at	VIL2	Villin 2 (ezrin)	6q25.2-q26	cellular morphogenesis, cytoskeletal anchoring
215211_at	---	Clone 23832 mRNA sequence	---	---
215308_at	G22P1	Thyroid autoantigen 70kDa (Ku antigen)	22q13.2-q13.31	DNA ligation, DNA repair, double-strand break repair via nonhomologous end-joining,
				DNA recombination, positive regulation of transcription, DNA-dependent
215311_at	---	MRNA full length insert cDNA clone EUROIMAGE 21920	---	---
215312_at	---	DNA damage repair and recombination protein RAD52 pseudogene	---	---
215402_at	APPBP2	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	17q21-q23	intracellular protein transport
215435_at	DDEF1	Development and differentiation enhancing factor 1	8q24.1-q24.2	regulation of GTPase activity
215554_at	GPLD1	glycosylphosphatidylinositol specific phospholipase D1	6p22.3-p22.2	cell-matrix adhesion
215645_at	FLCN	Hypothetical protein MGC13008	17p11.2	---
215657_at	SLC26A3	Solute carrier family 26, member 3	7q31	transport, anion transport, excretion, sulfate transport
215759_at	FLJ12056	hypothetical protein FLJ12056	2p13.3	---
215844_at	TNPO2	transportin 2 (importin 3, karyopherin beta 2b)	19p13.13	protein-nucleus import, docking, protein transport
215850_s_at	NDUFA5	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	7q32	electron transport
215911_x_at	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3	Xq28	cation transport, calcium ion transport, metabolism
215965_at	---	CDNA FLJ12359 fis, clone MAMMA1002355	---	---
216003_at	CDRT1	CMT1A duplicated region transcript 1	17p12	---
216132_at	ASTN2	Astrotactin 2	9q33.1	---
216165_at	---	CDNA: FLJ21997 fis, clone HEP06590	---	---
216201_at	---	CDNA: FLJ21586 fis, clone COL06920	---	---
216225_at	ANTXR1	Anthrax toxin receptor 1	2p13.1	---
216256_s_at	SERPINB13	serine (or cysteine) proteinase inhibitor,	18q21.3-q22	response to UV, regulation of proteolysis and peptidolysis
		clade B (ovalbumin), member 13		
216280_s_at	DICER1	Dicer1, Dcr-1 homolog (Drosophila)	14q32.13	RNA processing, RNA interference, targeting of mRNA for destruction
216329_at	---	---	---	---
216419_at	CROCC	ciliary rootlet coiled-coil, rootletin	1pter-p36.11	---
216420_at	---	---	---	---
216474_x_at	TPSAB1/2	tryptase alpha/beta , tryptase beta 2	16p13.3	proteolysis and peptidolysis, defense response, proteolysis and peptidolysis
216577_at	---	---	---	---
216596_at	LOC440265	similar to DKFZP434L187 protein	15q13.3	---
216740_at	TRERF1	Transcriptional regulating factor 1	6p21.1-p12.1	regulation of transcription, DNA-dependent, steroid biosynthesis, cholesterol catabolism, development,
				homeostasis, positive regulation of transcription, DNA-dependent, regulation of hormone biosynthesis
216782_at	KCNJ15	Potassium inwardly-rectifying channel, subfamily J, member 15	21q22.2	ion transport, potassium ion transport
216801_at	LOC400742	hypothetical gene supported by BC033316	1p36.13	---
216815_at	---	---	---	---
216856_s_at	---	---	---	---
216948_at	---	---	---	---
217363_x_at	---	---	---	---
217386_at	---	---	---	---
217395_at	MT4	metallothionein IV	16q13	---
217452_s_at	B3GALT2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	1q31	protein amino acid glycosylation
217504_at	ABCA6	ATP-binding cassette, sub-family A (ABC1), member 6	17q24.3	transport
217518_at	FER1L3	fer-1-like 3, myoferlin (C. elegans)	10q24	muscle contraction, circulation
217539_at	C18orf25	chromosome 18 open reading frame 25	18q21.1	---
217566_s_at	TGM4	transglutaminase 4 (prostate)	3p22-p21.33	peptide cross-linking, protein amino acid polyamination
217588_at	CATSPER2	cation channel, sperm associated 2	15q14	cation transport
217685_at	SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters),	17q25	transport, organic anion transport, monocarboxylic acid transport
		member 3		
217688_at	---	---	---	---
218890_x_at	MRPL35	mitochondrial ribosomal protein L35	2p11.2	---
218978_s_at	MSCP	mitochondrial solute carrier protein	8p21.2	transport
219203_at	C14orf122	chromosome 14 open reading frame 122	14q11.2	---
219312_s_at	ZBTB10	zinc finger and BTB domain containing 10	8q13-q21.1	transcription, regulation of transcription, DNA-dependent
219408_at	PRMT7	protein arginine N-methyltransferase 7	16q22.1	---
219780_at	LOC51333	mesenchymal stem cell protein DSC43	16p11.2	---
219789_at	NPR3	natriuretic peptide receptor C/guanylate cyclase C	5p14-p13	skeletal development
		(atrionatriuretic peptide receptor C)		
219903_s_at	CYP2C8	cytochrome P450, family 2, subfamily C, polypeptide 8	10q23.33	electron transport, electron transport, transport
219984_s_at	HRASLS	HRAS-like suppressor	3q29	---
220070_at	FLJ13798	hypothetical protein FLJ13798	16p12.1	---
220077_at	FLJ22349	hypothetical protein FLJ22349	22q13.2	---
220084_at	C14orf105	chromosome 14 open reading frame 105	14q22.3	---
220178_at	C19orf28	chromosome 19 open reading frame 28	19p13.3	---
220180_at	SE57-1	CTCL tumor antigen se57-1	18q21	---
220185_at	SPTBN4	spectrin, beta, non-erythrocytic 4	19q13.13	cytoskeletal anchoring, cytoskeletal anchoring, vesicle-mediated transport, vesicle-mediated transport
220223_at	FLJ12735	hypothetical protein FLJ12735	17q11.2	protein folding
220258_s_at	FLJ10385	hypothetical protein FLJ10385	17p13.1	---
220270_at	TDRD4	tudor domain containing 4	13q12.12	---
220382_s_at	ARHGAP28	Rho GTPase activating protein 28	18p11.23	viral release
220411_x_at	FLJ23447	hypothetical protein FLJ23447	19p13.12	---
220420_at	LMAN1L	lectin, mannose-binding, 1 like	15q24.1	---
220448_at	KCNK12	potassium channel, subfamily K, member 12	2p22-p21	ion transport, potassium ion transport
220471_s_at	MYCT1	myc target 1	6q25.2	---
220587_at	---	---	---	---
220920_at	ATP10B	ATPase, Class V, type 10B	5q34	cation transport
220938_s_at	GMEB1	glucocorticoid modulatory element binding protein 1	1p35.3	---
221091_at	INSL5	insulin-like 5	1p31.1-p22.3	physiological process
221136_at	GDF2	growth differentiation factor 2	10q11.22	growth
221183_at	---	CDNA: FLJ23604 fis, clone LNG15857	---	---
221233_s_at	KIAA1411	KIAA1411	6q12-q13	---
221487_s_at	ENSA	endosulfine alpha	1q21.2	transport, response to nutrients
221681_s_at	DSPP	dentin sialophosphoprotein	4q21.3	ossification, cell adhesion, development, perception of sound
221713_s_at	FLJ12748	hypothetical protein FLJ12748	3q27.1	---
221968_s_at	LOC51333	Mesenchymal stem cell protein DSC43	16p11.2	---
222074_at	UROD	uroporphyrinogen decarboxylase	1p34	heme biosynthesis
222135_at	---	Similar to Hypothetical zinc finger protein KIAA1958	19q13.43	---
222202_at	---	CDNA FLJ14293 fis, clone PLACE1007856	---	---
222246_at	STXBP3	Syntaxin binding protein 3	1p13.3	vesicle docking during exocytosis, protein transport, vesicle-mediated transport
222269_at	CXorf33	chromosome X open reading frame 33	Xq21.1	---
222383_s_at	ALOXE3	arachidonate lipoxygenase 3	17p13.1	electron transport, leukotriene biosynthesis
Tab. A6: IVIG-specific genes				
Subtraction of IVMP-specific genes after treatment of IVIG-specific genes after treatment according to parametric t-test;				
Probe-sets listed above are specifically differentially regulated upon IVIG therapy;				
Criteria for IVIG treatment: a minimum of a 2-fold change in 100% of patients;				
Criteria for IVMP treatment: a minimum of a 2-fold change in 100% of patients;				
	immune or inflammatory response			
	proliferation, cell cycle			
	apoptosis			
	signalling			
	transcription			

5.2 Publications

5.2.1

Mechanisms of action of intravenous immunoglobulins (IVIG) in patients with relapsing-remitting Multiple Sclerosis

N.Pigard, H.Kuusisto, I.Elovaara, R. Paalavuo, H.P. Scharz, B.Reipert

will be submitted to the Journal of Allergy and Clinical Immunology in November 2005;

5.3 Posters

5.3.1

N.Pigard, H.Kuusisto, I.Elovaara, R. Paalavuo, K. Zimmermann, H.P. Scharz, B.Reipert (2004): **Mechanisms of action of intravenous immunoglobulins in patients with relapsing-remitting multiple sclerosis**

Poster presentation at the 14th Meeting of the European Neurological Society, 26-30 June 2004, Barcelona, Spain

Journal of Neurology, Volume 251, Suppl.3, June 2004: Poster 723

5.3.2

Pigard N, Kuusisto H, Elovaara I, Paalavuo R, Zimmermann K, Schwarz HP and Reipert BM (2004): **Gene expression profiles of peripheral T cells in patients with relapsing-remitting multiple sclerosis after treatment with intravenous immunoglobulins (IVIG)**

Poster presentation at the 8th Congress of the European Federation of Neurological Societies (EFNS), September 2004, Paris, France

Eur J Neurol 2004; 11(suppl 2): P2437

5.3.3

Pigard N, Kuusisto H, Elovaara I, Paalavuo R, Zimmermann K, Schwarz HP and Reipert BM (2004): **Intravenous immunoglobulin (IVIG) in patients with Relapsing-Remitting Multiple Sclerosis – towards a better understanding of the mechanism of action**

Poster presentation at the Annual meeting of the Austrian Society for Allergology and Immunology (ÖGAI), December 2004, Vienna, Austria

5.3.4

Effects of intravenous immunoglobulins (IVIg) treatment of patients with relapsing-remitting multiple sclerosis on gene expression profiles of their peripheral t cells

Abstract submitted for Poster presentation at the 9th Congress of the European Federation of Neurological Societies (EFNS), September 2005, Athens, Greece

the abstract will be published in the European Journal of Neurology;

5.3.5

N. Pigard, H. Kuusisto, R. Paalavuo, I. Elovaara, H.P. Schwarz, B. Reipert; BMT Research, Tampere University Hospital, Baxter Bioscience (Vienna, A; Tampere, FIN): **Differentially expressed genes in peripheral T cells obtained from patients with relapsing-remitting multiple sclerosis after treatment with intravenous immunoglobulins**

Poster presentation at the 21th Congress of the European Committee / 10th Annual Meeting of the Americas Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), taking place in Thessaloniki, 28 September - 1 October 2005;

Mechanisms of action of intravenous immunoglobulins (IVIG) in patients with relapsing-remitting multiple sclerosis

Pigard Nadine[1], Kuusisto Hanna[2], Elovaara Irina[2], Zimmermann Klaus[3], Schwarz Hans-Peter[1,3], Reipert Birgit[1,3]

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INTRODUCTION

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of inflammatory and autoimmune diseases of the central nervous system including multiple sclerosis (MS).
Although IVIGs appear to have substantial short- and long-term therapeutic potential in these diseases, the mechanisms underlying these effects are not elucidated.
Recent studies suggest that a modulation of T-cell responses is involved in the immunmodulatory activities of IVIG in MS (1) (2). Therefore, we asked the question whether certain gene expression profiles in T cells obtained from patients with MS undergoing IVIG treatment correlate with the therapeutic effectiveness of IVIG.

AIM OF THE STUDY

Identification and characterization of genes expressed in $CD4^+/CD8^+$ PBMC obtained from patients with Relapsing Remitting MS (RRMS) in acute exacerbation before and after treatment with IVIG.

METHODS

1. Patients included in the study

Inclusion criteria:
- clinically or laboratory-supported definite RRMS in acute exacerbation
- Expanded Disability Status Scale (EDSS) 0-5.5
- age 18 and 55 years

Exclusion criteria:
- Patients with prior treatment
  - immunosuppressants within 9 month
  - corticosteroids 8 weeks
  - acute exacerbation within 8 weeks before entry
- Severe concurrent disease

2. Treatment of patients

All 10 patients included in the study received a 5-day course of 0.4g/kg bodyweight Endobulin S/D (Baxter AG, Vienna, Austria) per day. Treatment of patients as well as clinical evaluation was done at the Department of Neurology, Tampere University Hospital, Tampere, Finnland.

3. Blood sampling

Blood samples were taken immediately before the first dose of IVIG as well as 24 hours and three weeks after the last dose.

4. Clinical examination

- Interview
- EDSS
- MRI of brain and spinal cord
- Timing of clinical examination:
  - before first dose of IVIG
  - 24 h after last dose of IVIG
  - 3 weeks after last dose of IVIG

5. Preparation of $CD4^+/CD8^+$ PBMC

Peripheral blood mononuclear cells (PBMC) were prepared within 60 min after blood sampling. T cells were positively isolated from PBMC at 4°C using a mixture of non-stimulating anti-CD4 and anti-CD8 Dynabeads (Dynal, Oslo, Norway).
Purity of separated cells was confirmed by FACS-analysis.

6. Preparation of RNA and gene chip analysis

Purified cells were immediately transferred into TRIzol (Gibco/Invitrogen,Carlsbad, California) and stored at -80°C until preparation of RNA samples. 5µg of total RNA were in vitro-transcribed using the Affymetrix Eukaryotic Target Protocol. The cRNA was labeled with streptavidin-phycoerythrin conjugate and hybridized to the Human U133-A Genechip (Affymetrix).

7. Data analysis

Data obtained from the processing of the chips were analyzed with GCOS-Software (Affymetrix) and EPClust (http://ep.ebi.ac.uk/EP/EPCLUST/index.cgi)
We searched for genes that were up- or down-regulated ≥ 2-fold and were found in at least 40% of the patients (4 out of 10). Using the EPClust programme we grouped differentially regulated genes in clusters using the signal log ratios.

RESULTS

1. Therapeutic effectiveness of IVIG treatment

The EDSS score was significantly reduced in patients after IVIG-treatment (table 1). Data obtained in MRI analysis correlated with the improvement of the EDSS score.

Table 1: Clinical outcome: EDSS score before and after IVIG treatment

EDSS in stable phase	EDSS in acut exertion before IVIG	EDSS 3 weeks after IVIG treatment
2.42 ± 0.29	3.83 ± 0.32**	2.8 ± 0.25**

** p<0.01

2. Purity of $CD4^+/CD8^+$ PBMC cells after magnetic bead separation

Using FACS-analysis we could show that the majority of cells isolated from PBMC were T cells. Most of the other lymphoid cells were depleted, including $CD19^+$ cells, $CD11b^+$ and $CD56^+$ cells.
Almost all non-T cells ($CD3^-$ cells) were $CD14^+$ cells. FACS-analysis showed a proportion of 75% $CD3^+$ T cells and a proportion of 18% $CD14^+$ non- T cells (Fig. 1).

3. Gene expression analysis

Microarray-analysis revealed a number of differentially expressed genes after IVIG treatment (table2). Table 3 contains a complete list of all genes that were at least 2-fold up-or down-regulated in expression in at least 50% of all patients.

Figure 1
Purity of isolated cells: FACS-analysis using anti-CD3-FITC (FL1-H) and anti-CD14-PE (FL2-H) antibodies

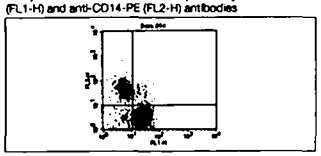


Table 2: Number of genes in $CD4^+/CD8^+$ PBMC of RRMS patients with at least 2-fold change in gene expression.

Fold change in gene expression	Number of genes affected		
	A	B	C
≥ 2-fold change in any patient	2585	4682	3455
≥ 2-fold change in at least 40% of patients	33	7	134
≥ 2-fold change in at least 50% of patients	3	1	43
≥ 2-fold change in at least 60% of patients	0	0	11

A: 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy
B: 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy
C: 24 hours compared to three weeks after completion of IVIG therapy

4. Analysis of gene clusters

Euclidean distance/K-means clustering of all genes differentially regulated in at least 40% of the patients revealed 2 main effects of IVIG on gene expression (Fig. 2), namely up-regulation and down-regulation of certain genes.

Genes that were up-regulated upon IVIG treatment could be clustered into 3 sub-groups:
- genes up-regulated at 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment, remained up-regulated at 3 weeks after completion of IVIG therapy compared to day 0 (7 genes)
- genes up-regulated at 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment, returned to baseline-levels at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (14 genes)
- genes up-regulated at 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment, were down-regulated below baseline-level at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (23 genes)

Genes that were down-regulated upon IVIG treatment could be clustered into 3 subgroups:
- genes down-regulated at 3 weeks compared to 24 hours after completion of IVIG therapy (126 genes)
- genes down-regulated at 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment - some of these genes reached baseline-levels after 3 weeks, the majority of genes remained down-regulated until 3 weeks after completion of IVIG therapy (8 genes)

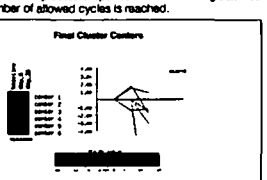
Table 3: Genes differentially expressed in $CD4^+/CD8^+$ PBMC of RRMS-patients

Upregulated/ Downregulated genes at 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
221841_s_at	↑	KLF4	Kruppel-like factor 4 (gut)
204439_at	↑	C1orf29	chromosome 1 open reading frame 29
214084_x_at	↑		Homo sapiens sorbitol to Neutrophil cytosolic factor 1 (LOC378112), mRNA
Upregulated/ Downregulated genes at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
204619_s_at	↓	CSPG2	chondroitin sulfate proteoglycan 2 (versican)
Upregulated/ Downregulated genes at 24 hours compared to 3 weeks after completion of IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
201422_at	↓	IFI30	interferon, gamma-inducible protein 30
203561_at	↓	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor for (CD32)
204445_s_at	↓	ALOX5	electron transporter/antioxidant/oxidoreductase/oxidative biosynthesis
204533_at	↓	CXCL10	cell motility/chemotaxis/inflammatory response/immune response/cell surface receptor linked signal transduction
207697_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
210146_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
211100_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
211101_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
214388_s_at	↓	ALOX5	arachidonate 5-lipoxygenase
214511_x_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
216950_s_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
219519_s_at	↓	SN	sialoadhesin
220146_at	↓	TLR7	toll-like receptor 7
201669_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
201670_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
205119_s_at	↓	FPR1	formyl peptide receptor 1
208891_at	↓	DUSP6	dual specificity phosphatase 6
208892_s_at	↓	DUSP6	dual specificity phosphatase 6
208893_s_at	↓	DUSP6	dual specificity phosphatase 6
221841_s_at	↓	KLF4	Kruppel-like factor 4 (gut)
204858_s_at	↓	EOGF1	endothelial cell growth factor 1 (platelet-derived)
204961_s_at	↓	NCF1	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)
207610_s_at	↓	EMR2	egf-like module containing, mucin-like, hormone receptor-like 2
208018_s_at	↓	HCK	hemopoietic cell kinase
209189_at	↓	FGS	v-fos FBJ murine osteosarcoma viral oncogene homolog
212099_at	↓	ARHB	ras homolog gene family, member B
217763_s_at	↓	RAB31	RAB31, member RAS oncogene family
219788_at	↓	PILRA	paired immunoglobulin-like type 2 receptor alpha
220088_at	↓	C5R1	complement component 5 receptor 1 (C5a ligand)
201380_at	↓	CST3	cystatin C (amyloid angiopathy and cerebral hemorrhage)
201798_s_at	↓	FER1L3	fer-1-like 3, myoferlin (C. elegans)
202510_s_at	↓	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
202833_s_at	↓	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
205638_at	↓	HK3	hexokinase 3 (white cell)
206488_s_at	↓	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)
208890_s_at	↓	PLXNB2	plexin B2
210423_s_at	↓	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
210879_x_at	↓	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
211429_s_at	↓		Homo sapiens PRO2275 mRNA, complete cds
213418_at	↓	HSPA6	heat shock 70kDa protein 6 (HSP70B)
213472_at	↓	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)
218559_s_at	↓	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)

Genes involved in immune response are underlined in yellow;
Genes involved in signal transduction are underlined in lavender;
Genes involved in cell cycle are underlined in blue.
Genes involved in cell motility are underlined in orange;
Genes involved in other biological processes are underlined in grey;
Genes present in 60% of the patients are marked in red

- genes down-regulated at 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment, remained down-regulated until 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (48 genes);

Figure 2
Euclidean distance / k-means clustering of differentially regulated genes at 24 hours (day 5) and 3 weeks (day 26) after IVIG treatment, compared to day 0 before IVOG treatment (baseline)
The K-means procedure assigns all genes to their respective clusters (the cluster to which centers they are closest) and refines cluster centers to be the geometric centers of gravity for each defined cluster. This process is repeated until the clustering stabilizes or the number of allowed cycles is reached.



DISCUSSION

The mechanisms of action of IVIG in autoimmune-disorders like MS is still poorly understood. We investigated the impact of human IVIG on gene-expression profiles in $CD4^+/CD8^+$ PBMC obtained from RRMS patients in exacerbation. This cell mixture contained both T cells and monocytes. The enrichment of monocytes together with T cells was due to the fact that monocytes express the CD4 antigen. Microarray data revealed a number of 176 genes that were differentially expressed in at least 40% of all patients after treatment with IVIG. 47 of these genes were expressed in at least 50% of the patients. The identified genes are known to be involved in different biological processes such as immune responses, signal transduction, cell cycle and cell motility. All these physiological processes might be important for the therapeutic efficiency of IVIG in the treatment of acute exacerbations in MS.
Currently we perform Real-Time PCR to confirm changes in gene expression.

The clinical outcome of the study showed that IVIG given in a 5-day course at 0.4g/kg per day was effective and caused a change in EDSS score from baseline levels (day 0 before IVIG treatment) to week 3 after IVIG treatment as well as changes in the volume and number of several MRI measures. Furthermore, the treatment was safe and well tolerated.

REFERENCES

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2. Modiano et al., 1997: J Immunol Immunpathol 83 (1): 77-85

Gene expression profiles of peripheral T-cells in patients with relapsing-remitting multiple sclerosis after treatment with intravenous immunoglobulins (IVIG)

Pigard Nadine[1], Kuusisto Hanna[2], Elovaara Irina[2], Raija Paalavuo[2], Zimmermann Klaus[3], Schwarz Hans-Peter[1,3], Reipert Birgit[1,3]

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[3]Baxter BioScience, Vienna, Austria

INTRODUCTION

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of inflammatory and autoimmune diseases of the central nervous system including multiple sclerosis (MS).
Although IVIGs appear to have substantial short- and long-term therapeutic potential, the mechanisms underlying these effects are not elucidated.
Recent studies suggested a modulation of T-cell response is involved in the immunomodulatory activities of IVIG in MS (1) (2).

AIM OF THE STUDY

Identification and characterization of genes expressed in T cells that might be involved in the immunomodulatory activity of IVIG in the treatment of exacerbations in Relapsing Remitting MS (RRMS).

METHODS

1. Patients included in the study

Inclusion criteria:
- clinically or laboratory-supported definite RRMS in acute exacerbation
- Expanded Disability Status Scale (EDSS) 0-5.5
- age 18 and 55 years

Exclusion criteria:
- Patients with prior treatment
  - immunosuppressants within 9 month
  - corticosteroids 8 weeks
  - acute exacerbation within 8 weeks before entry
- Severe concurrent disease

2. Treatment of patients

All 10 patients included in the study received a 5-day course of 0.4g/kg bodyweight Endobulin S/D (Baxter AG, Vienna, Austria) per day.
Treatment of patients as well as clinical evaluation was done at the Department of Neurology, Tampere University Hospital, Tampere, Finnland.
Patients were also subjected to MRI diagnosis of brain and spinal cord before and three weeks after IVIG treatment.

3. Blood sampling

Blood samples were taken immediately before the first dose of IVIG as well as 24 hours and three weeks after the last dose.

4. Clinical examination

- Interview
- EDSS
- MRI of brain and spinal cord
- Timing of clinical examination:
  - before 5 day course of IVIG
  - 24 h after course
  - 3 weeks after course

5. Preparation of T cells

Peripheral blood mononuclear cells (PBMC) were prepared within 60 min after blood sampling. T cells were positively isolated from PBMC at 4°C using a mixture of non-stimulating anti-CD4 and anti-CD8 Dynabeads (Dynal, Oslo, Norway).
Purity of T cells was confirmed by FACS-Analysis.

6. Preparation of RNA and gene chip analysis

After preparation, T cells were immediately transferred into TRIzol (Gibco/Invitrogen,Carlsbad, California) and stored at -80°C until preparation of RNA samples. 5µg of total RNA were invitro-transcribed using the Affymetrix Eurkaryotic Target Protocol, the cRNA was labeled with streptavidin-phycoerythrin conjugate and hybridized to the Human U133-A Genechip (Affymetrix).

7. Data analysis

Data obtained from the processing of the chips were analyzed with GCOS-Software (Affymetrix) and EPClust (http://ep.ebi.ac.uk/EP/EPCLUST/index.cgi).
We searched for genes that were up- or down-regulated ≥ 2-fold and were found in at least 40% of the patients (4 out of 10).
Using the EPClust programme we grouped differentially regulated genes in clusters using the signal log ratios.

RESULTS

1. Therapeutic effectiveness of IVIG treatment

Monitoring of RRMS patients regarding the clinical data, especially the EDSS score which was significantly reduced after IVIG-treatment, and MRI analysis (data not shown) did show a therapeutic effect of IVIG.

Table 1: Clinical outcome: Change in EDSS score

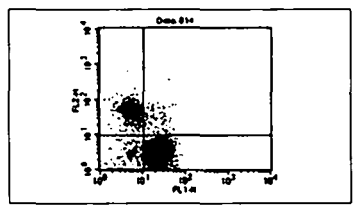
EDSS in stable phase	EDSS before IVIG	EDSS at 3 weeks
2.42 ± 0.28	3.83 ± 0.32**	2.6 ± 0.25**

** p<0.01

2. Purity of T cells after magnetic bead separation

Using Facs-Analysis we could show that the majority of cells isolated
from PBMC were purified T cells with depletion of most other lymphoid cells including $CD19^-$ cells, $CD11b^-$ and $CD56^-$ cells.
Almost all $CD3^+$ cells are $CD14^-$ cells (Fig. 1). FACS-Analysis using CD3-FITC and CD14-PE dye showed a proportion of 75% $CD3^+$ cells, a proportion of 15.6% $CD14^+$ cells and 2.7% double-positive $CD3^+/CD14^+$ cells. The remaining 6.8% may be due to unspecific binding of the antibodies to various Fc-Receptors.

Figure 1
Purity of T cells: FACS-Analysis using CD3-FITC and CD14-PE



3. Gene expression analysis

Microarray-Analysis revealed a number of differentially expressed genes after IVIG treatment (Table2).

Table 2: Number of genes in $CD4^+$ or $CD8^+$ PBMC of RRMS patients with at least 2-fold change in gene expression.

Fold change in gene expression	Number of genes affected		
	A	B	C
≥ 2-fold change in any patient	2605	4602	3855
≥ 2-fold change in at least 40% of patients	35	7	134
≥ 2-fold change in at least 50% of patients	3	1	43
≥ 2-fold change in at least 60% of patients	0	0	11

Legend:
A. 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy
B. 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy
C. 3 weeks after completion of IVIG therapy compared to 24 hours after completion of IVIG therapy

4. Analysis of gene clusters

Euclidean distance/K-means clustering of all genes differentially regulated in at least 40% of the patients revealed 2 main effects of IVIG on gene expression (Fig. 2).

Genes that are up-regulated upon IVIG treatment can be clustered into 3 sub-groups:
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and remained up-regulated at 3 weeks after completion of IVIG therapy compared to day 0 (7 genes)
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and returned to baseline-levels at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (14 genes)
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and down-regulated below baseline-level at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (23 genes)

Genes that are down-regulated upon IVIG treatment can also be clustered into 3 subgroups:
- genes were down-regulated regulated 3 weeks after IVIG therapy compared to 24 hours after completion of IVIG therapy (130 genes)
- genes were down-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and partly reach baseline-level again after 3 weeks, whereas the majority remains down-regulated until 3 weeks after completion of IVIG therapy compared to day 0 (6 genes)

Figure 2
2 Euclidean distance / k-means clustering of differentially regulated genes after 6 days and 26 days of IVIG treatment:
The K-means procedure assigns all genes to their respective clusters (the cluster to whose center they are closest) and refines cluster centers to be the geometric centers of gravity for each defined cluster. This process is repeated until the clustering stabilizes or the number of allowed cycles is reached.

The following table contains a complete list of all genes that were at least 2-fold up-or down-regulated in expression in at least 50% of all patients.

Table 3: Genes differentially expressed in $CD4^+$ and $CD8^+$ PBMC of RRMS-patients

Upregulated/ Downregulated genes at 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
221841_s_at	↑	KLF4	Kruppel-like factor 4 (gut)
204439_at	↑	C1orf29	chromosome 1 open reading frame 29
214084_x_at	↑		Homo sapiens similar to Neutrophil cytosolic factor 1 (LOC378117), mRNA
Upregulated/ Downregulated genes at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
204619_s_at	↓	CSPG2	chondroitin sulfate proteoglycan 2 (versican)
Upregulated/ Downregulated genes at 24 hours compared to 3 weeks after completion of IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
201422_at	↓	IFI30	interferon, gamma-inducible protein 30
203561_at	↓	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor for (CD32)
204445_s_at	↓	ALOX5	electron transport/inflammatory response/leukotriene biosynthesis
204533_at	↓	CXCL10	cell motility/chemotaxis/inflammatory response/immune response/cell surface receptor linked signal transduction
207697_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
210146_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
211100_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
211101_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
214366_s_at	↓	ALOX5	arachidonate 5-lipoxygenase
214511_x_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
216950_s_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
219519_s_at	↓	SN	sialoadhesin
220146_at	↓	TLR7	toll-like receptor 7
201669_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
201670_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
205119_s_at	↓	FPR1	formyl peptide receptor 1
208891_at	↓	DUSP6	dual specificity phosphatase 6
208892_s_at	↓	DUSP6	dual specificity phosphatase 6
208893_s_at	↓	DUSP6	dual specificity phosphatase 6
221841_s_at	↓	KLF4	Kruppel-like factor 4 (gut)
204858_s_at	↓	EOGF1	endothelial cell growth factor 1 (platelet-derived)
204961_s_at	↓	NCF1	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)
207610_s_at	↓	EMR2	egf-like module containing, mucin-like, hormone receptor-like 2
208018_s_at	↓	HCK	hemopoietic cell kinase
209189_at	↓	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
212099_at	↓	ARHB	ras homolog gene family, member B
217763_s_at	↓	RAB31	RAB31, member RAS oncogene family
219788_at	↓	PILRA	paired immunoglobin-like type 2 receptor alpha
220088_at	↓	C5R1	complement component 5 receptor 1 (C5a ligand)
201360_at	↓	CST3	cystatin C (amyloid angiopathy and cerebral hemorrhage)
201798_s_at	↓	FER1L3	fer-1-like 3, myoferlin (C. elegans)
202510_s_at	↓	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
202833_s_at	↓	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
205936_s_at	↓	HK3	hexokinase 3 (white cell)
206488_s_at	↓	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)
208890_s_at	↓	PLXNB2	plexin B2
210423_s_at	↓	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
210873_s_at	↓	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
211429_s_at	↓		Homo sapiens PRO2275 mRNA, complete cds
213418_at	↓	HSPA6	heat shock 70kDa protein 6 (HSP70B')
213472_at	↓	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)
218559_s_at	↓	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)

Genes involved in immune response are underlined in yellow.
Genes involved in signal transduction are underlined in lavender.
Genes involved in cell cycle are underlined in blue.
Genes involved in cell motility are underlined in orange.
Genes involved in other biological processes are underlined in grey.
Genes present in 60% of the patients are marked in red.

DISCUSSION

The mechanism of action of IVIG in autoimmune-disorders like MS is still poorly understood. The increasing efficacy of this immunomodulatory treatment requires to elucidate the mode of action underlying the pathogenesis of the disease.
In MS, auto-reactive T-cells which cross the blood-brain-barrier are the main target of demyelinating antibodies. Therefore we investigated the impact of human IVIG on gene-expression in T-cells of RRMS patients.
The clinical outcome of the study shows that IVIG given in a 5-day course of 0.4g/kg once daily was efficacious in the treatment of multiple sclerosis. It caused a change in EDSS score from baseline (before IVIG) to week 3 (after IVIG) as well as changes in the volume or number of several MRI measures.
The treatment also was safe and well tolerated.
Therfore IVIG is a valuable alternative for treatment of acute exacerbations in multiple sclerosis.

For gene expression analysis we used a mixture of $CD4^+$ and $CD8^+$ PBMC and Monocytes, as FACS Analysis gave evidence that most of the CD3- cells primarily consist of $CD14^+$ cells.
Microarray-data revealed a number of 176 genes differentially expressed in at least 40% of all patients after treatment with IVIG, including 47 genes that were even expressed in at least 50% of the patients. These genes are involved in different biological processes, including immune response, signal transduction, cell cycle and cell motility as well as many other processes. Currently we perform Real-Time PCR to confirm these changes in gene expression.
All these physiological processes might be important for the therapeutic efficiency of IVIG in the treatment of acute exacerbations in MS.

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Intravenous immunoglobulin (IVIG) in patients with Relapsing-Remitting Multiple Sclerosis - towards a better understanding of the mechanism of action

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INTRODUCTION

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of inflammatory and autoimmune diseases of the central nervous system including multiple sclerosis (MS).
Although IVIGs appear to have substantial short- and long-term therapeutic potential, the mechanisms underlying these effects are not elucidated.
Recent studies suggested a modulation of T-cell response is involved in the immunomodulatory activities of IVIG in MS (1) (2).

AIM OF THE STUDY

Identification and characterization of genes expressed in T cells that might be involved in the immunomodulatory activity of IVIG in the treatment of exacerbations in Relapsing Remitting MS (RRMS).

METHODS

1. Patients included in the study

Inclusion criteria:
- clinically or laboratory-supported definite RRMS in acute exacerbation
- Expanded Disability Status Scale (EDSS) 0-5.5
- age 18 and 55 years

Exclusion criteria:
- Patients with prior treatment
  - immunosuppressants within 9 month
  - corticosteroids 8 weeks
  - acute exacerbation within 8 weeks before entry
- Severe concurrent disease

2. Treatment of patients

All 10 patients included in the study received a 5-day course of 0.4g/kg bodyweight Endobulin S/D (Baxter AG, Vienna, Austria) per day.
Treatment of patients as well as clinical evaluation was done at the Department of Neurology, Tampere University Hospital, Tampere, Finnland.
Patients were also subjected to MRI diagnosis of brain and spinal cord before and three weeks after IVIG treatment.

3. Blood sampling

Blood samples were taken immediately before the first dose of IVIG as well as 24 hours and three weeks after the last dose.

4. Clinical examination

- Interview
- EDSS
- MRI of brain and spinal cord
- Timing of clinical examination:
  - before 5 day course of IVIG
  - 24 h after course
  - 3 weeks after course

5. Preparation of T cells

Peripheral blood mononuclerar cells (PBMC) were prepared within 60 min after blood sampling. T cells were positively isolated from PBMC at 4°C using a mixture of non-stimulating anti-CD4 and anti-CD8 Dynabeads (Dynal, Oslo, Norway).
Purity of T cells was confirmed by FACS-Analysis.

6. Preparation of RNA and gene chip analysis

After preparation, T cells were immediately transferred into TRIzol (Gibco/Invitrogen,Carlsbad, California) and stored at –80°C until preparation of RNA samples. 5µg of total RNA were invitro-transcribed using the Affymetrix Eurkaryotic Target Protocol, the cRNA was labeled with streptavidin-phycoerythrin conjugate and hybridized to the Human U133-A Genechip (Affymetrix).

7. Data analysis

Data obtained from the processing of the chips were analyzed with GCOS-Software (Affymetrix) and EPClust (http://ep.ebi.ac.uk/EP/EPCLUST/index.cgi).
We searched for genes that were up- or down-regulated ≥ 2-fold and were found in at least 40% of the patients (4 out of 10).
Using the EPClust programme we grouped differentially regulated genes in clusters using the signal log ratios.

RESULTS

1. Therapeutic effectiveness of IVIG treatment

Monitoring of RRMS patients regarding the clinical data, especially the EDSS score which was significantly reduced after IVIG-treatment, and MRI analysis (data not shown) did show a therapeutic effect of IVIG.

Table 1: Clinical outcome: Change in EDSS score

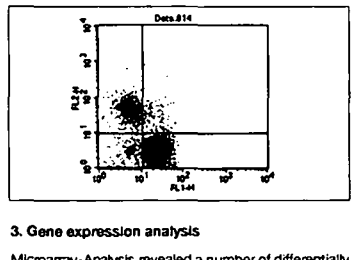
EDSS in stable phase	EDSS before IVIG	EDSS at 3 weeks
2.42 ± 0.28	3.83 ± 0.32**	2.6 ± 0.25**

** p<0.01

2. Purity of T cells after magnetic bead separation

Using Facs-Analysis we could show that the majority of cells isolated
from PBMC were purified T cells with depletion of most other lymphoid cells including $CD19^+$ cells, $CD11b^+$ and $CD56^+$ cells.
Almost all $CD3^+$ cells are $CD14^-$ cells (Fig. 1). FACS-Analysis using CD3-FITC and CD14-PE dye showed a proportion of 75% $CD3^+$ cells, a proportion of 15.6% $CD14^+$ cells and 2.7% double-positive $CD3^+/CD14^+$ cells. The remaining 6.8% may be due to unspecific binding of the antibodies to various Fc-Receptors.

Figure 1
Purity of T cells: FACS-Analysis using CD3-FITC and CD14-PE



3. Gene expression analysis

Microarray-Analysis revealed a number of differentially expressed genes after IVIG treatment (Table2).

Table 2: Number of genes in $CD4^+$ or $CD8^+$ PBMC of RRMS patients with at least 2-fold change in gene expression.

Fold change in gene expression	Number of genes affected		
	A	B	C
≥ 2-fold change in any patient	2605	4602	3855
≥ 2-fold change in at least 40% of patients	35	7	134
≥ 2-fold change in at least 50% of patients	3	1	43
≥ 2-fold change in at least 60% of patients	0	0	11

Legend:
A: 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy
B: 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy
C: 3 weeks after completion of IVIG therapy compared to 24 hours after completion of IVIG therapy

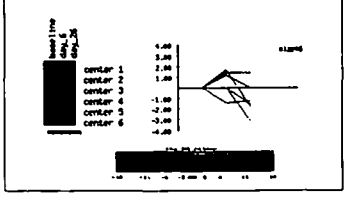
4. Analysis of gene clusters

Euclidean distance/K-means clustering of all genes differentially regulated in at least 40% of the patients revealed 2 main effects of IVIG on gene expression (Fig. 2).

Genes that are up-regulated upon IVIG treatment can be clustered into 3 sub-groups:
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and remained up-regulated at 3 weeks after completion of IVIG therapy compared to day 0 (7 genes)
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and returned to baseline-levels at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (14 genes)
- genes were up-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and down-regulated below baseline-level at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy (23 genes)

Genes that are down-regulated upon IVIG treatment can also be clustered into 3 subgroups:
- genes were down-regulated regulated 3 weeks after IVIG therapy compared to 24 hours after completion of IVIG therapy (130 genes)
- genes were down-regulated 24 hours after completion of IVIG therapy compared to day 0 before IVIG treatment and partly reach baseline-level again after 3 weeks, whereas the majority remains down-regulated until 3 weeks after completion of IVIG therapy compared to day 0 (6 genes)

Figure 2
2 Euclidean distance / k-means clustering of differentially regulated genes after 6 days and 26 days of IVIG treatment;
The K-means procedure assigns all genes to their respective clusters (the cluster to whose center they are closest) and refines cluster centers to be the geometric centers of gravity for each defined cluster. This process is repeated until the clustering stabilizes or the number of allowed cycles is reached.



The following table contains a complete list of all genes that were at least 2-fold up-or down-regulated in expression in at least 50% of all patients.

Table 3: Genes differentially expressed in $CD4^+$ and $CD8^+$ PBMC of RRMS-patients

Upregulated/Downregulated genes at 24 hours after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
221841_s_at	↑	KLF4	Kruppel-like factor 4 (gut)
204439_at	↑	C1orf29	chromosome 1 open reading frame 29
214084_x_at	↑		Homo sapiens similar to Neutrophil cytosolic factor 1 (LOC378112), mRNA
Upregulated/Downregulated genes at 3 weeks after completion of IVIG therapy compared to day 0 before IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
204619_s_at	↓	CSPG2	chondroitin sulfate proteoglycan 2 (versican)
Upregulated/Downregulated genes at 24 hours compared to 3 weeks after completion of IVIG therapy			
Probe Set ID	Expression	Gene Symbol	Gene Title
201422_at	↓	IFI30	interferon, gamma-inducible protein 30
203561_at	↓	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor for (CD32)
204445_s_at	↓	ALOX5	electron transportinflammatory responseleukotriene biosynthesis
204533_at	↓	CXCL10	cell motilitychemotaxisinflammatory responseimmune responsecell surface receptor linked signal transduction
207697_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
210146_x_at	↓	LILRB2	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
211100_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
211101_x_at	↓	LILRB1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1
214368_s_at	↓	ALOX5	arachidonate 5-lipoxygenase
214511_x_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
216950_s_at	↓	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
219519_s_at	↓	SN	sialoadhesin
220146_at	↓	TLR7	toll-like receptor 7
201669_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
201670_s_at	↓	MARCKS	myristoylated alanine-rich protein kinase C substrate
205119_s_at	↓	FPR1	formyl peptide receptor 1
208891_at	↓	DUSP6	dual specificity phosphatase 6
208892_s_at	↓	DUSP6	dual specificity phosphatase 6
208893_s_at	↓	DUSP6	dual specificity phosphatase 6
221841_s_at	↓	KLF4	Kruppel-like factor 4 (gut)
204858_s_at	↓	ECGF1	endothelial cell growth factor 1 (platelet-derived)
204961_s_at	↓	NCF1	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)
207610_s_at	↓	EMR2	egf-like module containing, mucin-like, hormone receptor-like 2
208018_s_at	↓	HCK	hemopoietic cell kinase
209189_at	↓	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
212099_at	↓	ARHB	ras homolog gene family, member B
217763_s_at	↓	RAB31	RAB31, member RAS oncogene family
219788_at	↓	PILRA	paired immunoglobin-like type 2 receptor alpha
220088_at	↓	C5R1	complement component 5 receptor 1 (C5a ligand)
201360_at	↓	CST3	cystatin C (amyloid angiopathy and cerebral hemorrhage)
201798_s_at	↓	FER1L3	fer-1-like 3, myoferlin (C. elegans)
202510_s_at	↓	TNFAIP2	tumor necrosis factor, alpha-induced protein 2
202833_s_at	↓	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
205936_s_at	↓	HK3	hexokinase 3 (white cell)
206488_s_at	↓	CD36	CD36 antigen (collagen type I receptor, thrombospondin receptor)
206890_s_at	↓	PLXNB2	plexin B2
210423_s_at	↓	SLC11A1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
210873_x_at	↓	APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
211429_s_at	↓		Homo sapiens PRO2275 mRNA, complete cds
213418_at	↓	HSPA6	heat shock 70kDa protein 6 (HSP70B')
213472_at	↓	HNRPH1	heterogeneous nuclear ribonucleoprotein H1 (H)
218559_s_at	↓	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)

Genes involved in immune response are underlined in yellow;
Genes involved in signal transduction are underlined in lavender;
Genes involved in cell cycle are underlined in blue;
Genes involved in cell motility are underlined in orange;
Genes involved in other biological processes are underlined in grey;
Genes present in 60% of the patients are marked in red

DISCUSSION

The mechanism of action of IVIG in autoimmune-disorders like MS is still poorly understood. The increasing efficacy of this immunomodulatory treatment requires to elucidate the mode of action underlying the pathogenesis of the disease.
In MS, auto-reactive T-cells which cross the blood-brain-barrier are the main target of demyelinating antibodies. Therefore we investigated the impact of human IVIG on gene-expression in T-cells of RRMS patients.
The clinical outcome of the study shows that IVIG given in a 5-day course of 0.4g/kg once daily was efficacious in the treatment of multiple sclerosis. It caused a change in EDSS score from baseline (before IVIG) to week 3 (after IVIG) as well as changes in the volume or number of several MRI measures.
The treatment also was safe and well tolerated.
Therfore IVIG is a valuable alternative for treatment of acute exacerbations in multiple sclerosis.

For gene expression analysis we used a mixture of $CD4^+$ and $CD8^+$ PBMC and Monocytes, as FACS Analysis gave evidence that most of the $CD3^-$ cells primarily consist of $CD14^-$ cells.
Microarray-data revealed a number of 176 genes differentially expressed in at least 40% of all patients after treatment with IVIG, including 47 genes that were even expressed in at least 50% of the patients. These genes are involved in different biological processes, including immune response, signal transduction, cell cycle and cell motility as well as many other processes. Currently we perform Real-Time PCR to confirm these changes in gene expression.
All these physiological processes might be important for the therapeutic efficiency of IVIG in the treatment of acute exacerbations in MS.

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Differentially expressed genes in peripheral T cells obtained from patients with relapsing-remitting multiple sclerosis after treatment with intravenous immunoglobulins (IVIG)

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BMT RESEARCH

INTRODUCTION

Intravenous immunoglobulins (IVIG) have been used successfully in the treatment of a number of inflammatory and autoimmune diseases of the central nervous system including multiple sclerosis (MS). Recent studies suggested that a modulation of
T-cell responses was involved in the immunmodulatory activities of IVIG in MS (1; 2). To explain the effects of IVIG in more detail, we studied gene expression profiles of peripheral T cells in patients with relapsing-remitting MS (RRMS) in exacerbation before and after treatment with IVIG. For comparison we studied patients who received intravenous methylprednisolone (IVMP)

PURPOSE OF THE STUDY

Identification of genes differentially expressed in peripheral T cells obtained from patients with relapsing remitting MS (RRMS) in acute exacerbation before and after treatment with IVIG.

METHODS

1. Patients included in the study

Inclusion criteria:
- clinically or laboratory-supported definite RRMS in acute exacerbation
- Expanded Disability Status Scale (EDSS) 0-5.5
- age 18 and 55 years

Exclusion criteria:
- prior treatment with.
  - immunosuppressants within 9 month
  - corticosteroids within 8 weeks
- acute exacerbation within 8 weeks before entry.
- severe concurrent disease

2. Treatment of patients

All 10 patients included in the study received a 5-day course of 0.4g/kg bodyweight Endobulin S/D (IVIG; Baxter AG, Vienna, Austria) per day. The control group of 5 patients received 1g/day intravenous methylprednisolon (IVMP). Treatment of patients as well as clinical evaluation was done at the Department of Neurology, Tampere University Hospital, Tampere, Finnland. The characteristics of patients included in the study are given in table 1.

Table 1. Characteristics of study population

Characteristics	patients treated with IVIG	patients treated with IVMP (controls)
Number of study population	10	5
Age average ± SD, year)	40 ± 10.6	35.3 ± 8.8
Sex (male vs female)	3 vs 7	0 vs 5
Disease duration (average ± SD, year)	5.6 ± 3.5	5.2 ± 3.6
Time current vs previous relapse (average ± SD, month)	17.6 ± 21.0	5 ± 3.2
EDSS score at remission (average ± SD, score)	3.7 ± 1.1	3.2 ± 2.4
EDSS score at acute relapse (average ± SD, score)	3.7 ± 1.1	4.2 ± 2.0)

3. Blood sampling

Blood samples were taken immediately before the first dose of IVIG or IVMP as well as on days 6 and 21 after the first dose.

4. Clinical examination

- Interview
- EDSS
- MRI of brain and spinal cord
- Timing of clinical examination.
  - before first dose of IVIG or IVMP
  - 6 days after the first dose of IVIG or IVMP
  - 3 weeks after first dose of IVIG or IVMP

5. Preparation of peripheral T cells

Penpheral blood mononuclerar cells (PBMC) were prepared within 60 min after blood sampling. T cells were isolated from PBMC at 4°C using a mixture of non-stimulating anti-CD4 and anti-CD8 Dynabeads (Dynal, Oslo, Norway).
Purity of separated cells was confirmed by FACS-analysis.

6. Preparation of RNA and gene chip analysis

Purified cells were immediately transferred into TRIzol (Gibco/Invitrogen,Carlsbad, California) and stored at –80° until preparation of RNA samples. 5µg of total RNA were in vitro-transcribed using the Affymetrix Eurkaryotic Target Protocol. The cRNA was labeled with streptavidin-phycoerythrin conjugate and hybridized to the Human U133-A Genechip (Affymetrix, Santa Clara, CA).

7. Data analysis

All data obtained were analyzed using a parametric t-test (Welch t-test with unpaired variances). Differences with p-values of less than 0.05 were considered to be statistically significant. Initially, all arrays were normalized for comparison between expression data obtained on day 0 (before treatment) and on days 6 and 21. All data were transformed logarithmically and all genes with a fold change ≥ 2 were included in the analysis.

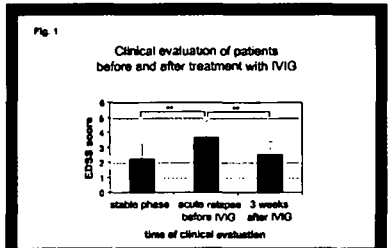
8. Real Time Polymerase chain reaction

The microarray data for 13 genes were confirmed by quantitative real-time PCR.
1µg of total T cell RNA was used for reverse transcription into cDNA according to the manufacturer's protocol (MBI Fermentas, Burlington, Canada).
100ng cDNA in 5µl nuclease-free water (Invitrogen, Carlsbad, CA) were quantitatively analyzed using different TaqMan® Assays-on-Demand (Applied Biosystems, Foster City, CA) with the ABPrism 7000 (Applied Biosystems). For normalization of expression data human GPDH and β-2 microglobulin were used as housekeeping genes.

RESULTS

1. Therapeutic effectiveness of IVIG treatment

The EDSS score was significantly reduced in patients after IVIG-treatment (Fig. 1). Data obtained in MRI analysis correlated with the improvement of EDSS scores (table 2). For comparison, MRI analysis in control patients who received IVMP treatment did not show significant improvement of disease activity (table 3).



Kurtzke's Expanded Disability Status Scale (EDSS) in patiens during stable phase and during acute relapse before and after treatment with IVIG.
** p<0.01

Table 2. MRI measurements of brain abnormalities before and after IVIG

Parameter	Before IVIG	After IVIG
Lesion vol cm³	mean ± SE	mean ± SE
T1	1. 76 ± 0.55	1.73 ± 0.59
T2	5.49 ± 1.09	5.08 ± 1.03*
Flair	15.76 ± 2.23	14.09 ± 1.94**
Gd-enhanced	0.32 ± 0.27	0.21 ± 0.24**
Brain volume	1124.94 ± 40.61	1120.31± 40.72
Gd+lesion N	2.83 ± 0.71	2.00 ± 0.60**
EDSS* score	3.8 ± 0.3	2.6 ± 0.2**

* p<0.05; ** p<0.01
* EDSS = Kurtzke's Expanded Disability Status Scale

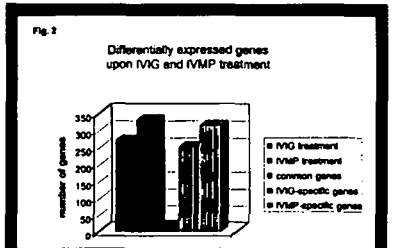
Table 3. MRI measurements of brain abnormalities before and after IVMP

Parameter	Before IVMP	After IVMP
Lesion vol cm³	mean ± SE	mean ± SE
T1	1.41 ± 0.60	1.64 ± 0.84
T2	11.15 ± 4.59	9.83 ± 4.17
Flair	24.37 ± 8.19	23.18 ± 8.05
Gd-enhanced	0.70 ± 0.39	0.63 ± 0.37
Brain volume	1056.32 ± 47.78	1045.07 ± 52.53
Gd+lesion N	3.0 ± 1.5	2.7 ± 1.4

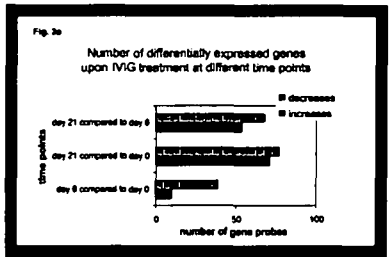
2. Differentially expressed genes after IVIG treatment

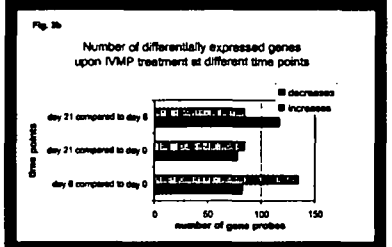
Microarray-analysis revealed a number of differentially expressed genes after IVIG treatment (Fig. 2). 271 genes were either up- or down-regulated. For comparison, 334 genes were differentially expressed in CD4⁺/CD8⁺ PBMC in patients after treatment with IVMP (Fig. 2). 18 genes were found to be differentially expressed in both groups of patients, leaving 253 genes to be differentially expressed specifically in patients after IVIG treatment (Fig. 2).

The kinetic distribution of differentially expressed genes differed between patients treated with IVIG and patients treated with IVMP (Fig. 3a and 3b). In patients treated with IVIG most differentially expressed genes were found on day 21 when compared to either day 0 or day 6 (Fig 3a).



Number of differentially expressed genes in CD4+/CD8+ PBMC obtained from patients upon treatment with IVIG or IVMP. Common genes were found in both groups of patients. IVIG-specific and IVMP-specific genes were only found in patients treated with IVIG or IVMP, respectively. All genes were at least 2-fold up- or down-regulated.





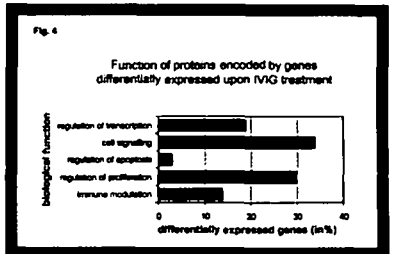
Number of differentially expressed genes at different time points during treatment with IVIG (Fig. 3a) or IVMP (Fig. 3b). All genes were at least 2-fold up- or down-regulated.

3. Confirmation of microarray data by Real Time Polymerase chain reaction (PCR)

Expression of 13 genes found to be differentially expressed using microarray analysis was investigated by Real Time PCR. Results obtained with the Real Time PCR showed the same trend of either up- or down-regulation as data obtained by microarray analysis.

4. Biological activity of proteins encoded by differentially expressed genes in patients after treatment with IVIG

Proteins encoded by most genes found to be differentially expressed in patients after treatment with IVIG are involved in either of the following: regulation of immune responses, regulation of proliferation, regulation of apoptosis, regulation of cell signaling or regulation of transcription (Fig. 4). Examples of proteins involved in regulation of immune responses are ligands for chemokine receptors, cytokines and proteins involved in biosynthesis or bioactivity of prostaglandines.



Biological function of proteins encoded by genes differentially expressed during IVIG therapy.

DISCUSSION AND CONCLUSIONS

This study was designed to identify genes differentially expressed in T cells of patients with RRMS in acute exacerbation after treatment with IVIG.
The clinical outcome of the study showed that IVIG given in a 5-day course at 0.4g/kg per day was effective and caused a significant change in EDSS score as well as changes in disease activity as analyzed by MRI. Furthermore, the treatment was safe and well tolerated.

We identified 253 genes differentially expressed specifically in patients after IVIG treatment. Most of the proteins encoded by these genes are known to be involved in the regulation of immune responses, cell proliferation, apoptosis, cell signaling and transcription. All these biological activities are likely to be involved in the regulation of disease activity in patients with RRMS. Therefore, we believe that we have identified a set of genes that might be associated with the biological activity of IVIG in patients with RRMS. Further evaluation of these genes in a larger group of patients will be necessary to confirm our data and to define a set of differentially expressed genes that correlate with the clinical efficacy of IVIG in RRMS.

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6. CURRICULUM VITAE

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