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Combination of immunosuppressive drugs leaves specific "fingerprint" on gene expression in vitro

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RESEARCH ARTICLE

Combination of immunosuppressive drugs leaves specific "fingerprint" on gene expression *in vitro*

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Abstract

Following organ transplantation many patients suffer from drug-related side effects, or receive more immunosuppression than necessary to prevent rejection. Hence, parameters are needed to tailor the immunosuppressive therapy to the individual needs of an organ recipient. The aim of this study was to determine whether drug combinations provoke specific gene expression patterns in a simple assay system *in vitro*. Stimulated peripheral blood lymphocytes were cultured in the presence of cyclosporine A, tacrolimus, mycophenolic acid, everolimus and sirolimus, or combinations thereof. Using a cDNA microarray, we found that all samples clustered in drug-specific groups. Gene expression profiles were almost identical in PBL treated with either cyclosporine A or tacrolimus, and with either sirolimus or everolimus. More than 50 genes were synergistically affected by combinations of calcineurin-inhibitors and TOR-inhibitors and drug-specific regulated genes could be identified for both substance groups. Our data suggest that *in vitro* gene profiling can be used to describe synergistic effects of immunosuppressive drugs. Furthermore, our approach may help to identify marker genes urgently needed to optimize and individualize immunosuppressive drug regimens after organ transplantation.

Introduction

Methods to analyze the response to drug combinations in a given individual are urgently needed in order to eventually develop protocols of individually "tailored" immunosuppression.⁽¹⁾ Recently it has been shown that in many cases synergistic effect of drugs *in vitro* correlates well with *in vivo* data. Furthermore, mRNA expression correlates well with protein expression levels and functional tests.^(2,3) Since effects of drugs are complex, the array technology is well suited to study impact of drugs without a priori knowledge of genes of interest.

It is well documented that different states of lymphocyte activation can be identified as "functional fingerprints" using microarray technology.⁽⁴⁾ There is evidence that gene expression patterns from PBL (peripheral blood lymphocytes) can predict presence of tolerogenic cells.⁽⁵⁾ Microarray-based gene expression profiles of organs have been shown to classify states of rejection in several models.⁽⁶⁻⁸⁾ Lamb et al. ⁽⁹⁾ showed that shared mechanisms of action can be deduced from gene expression data of *in vitro* experiments.

The aims of this study were to show that specific gene expression patterns of immunosuppressive drugs can be generated in a short *in vitro* experiment, and furthermore to identify specific or synergistic effects of the calcineurin-inhibitors cyclosporine A (CsA) and tacrolimus (FK), the TOR-inhibitors (target-of-rapamy-cin-inhibitors) everolimus (EVL) and sirolimus (SRL) and mycophenolic acid (MMF) or clinically used combinations thereof. For that purpose global gene expression of *in vitro* cultured PHA(phytohaemagglutinin) stimulated PBLs was monitored with 7.5k cDNA-microarrays.

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Materials and methods

Cell culture

PBL from three different healthy adults were isolated from whole blood samples using Ficoll density gradient centrifugation and cultured in RPMI medium (supplemented with fetal calf serum 10% and penicillin/ streptomycin 1%) in 12-well plates at 2×10^6 cells/well. After 24 hours PHA and immunosuppressive drugs were added to yield the following final concentration of drugs: PHA 1 µg/ml, CsA 50 ng/ml, FK 1 ng/ml, MMF 50 ng/ml, EVL 1 ng/ml, SRL 1 ng/ml and combinations of CsA or FK respectively with MMF or EVL or SRL. Drug dilutions were done in medium from stocks immediately before being added to culture. Parallel cultures were grown containing PHA only or PHA plus tween-ethanol or methanol at the same concentrations used for the drug dilutions, respectively.

Cells were harvested 24 h later, homogenized in 4 M guanidinium isothiocyanate with and 0.72% ß-mercaptoethanol. RNA was isolated in a cesiumchloride gradient instantaneously. After ethanol precipitation RNA was resuspended at a concentration of $0.4-4 \mu g/\mu l$ and stored at -80° C. We investigated 5 single drugs and 6 drug combinations, each repeated in a paired design by the same three biological replications. Three controls were done, one for each solvent condition. Additionally, we performed technical duplicates for one donor for incubations with two single drugs and their combination (complete with all controls) to compensate for lost pellets from another experiment. Finally, our study involved a total of 48 cDNA microarrays.

Hybridization

All hybridizations were performed in the presence of an equal amount of reference RNA (Stratagene, LaJolla, CA, USA) as recently described by Boldrick et al.⁽¹⁰⁾ In the presence of Cy3- labeled dUTP, $12 \mu g$ of PBL RNA were transcribed into cDNA using Stratascript reverse transcriptase (Stratagene, La Jolla, CA, USA). Likewise, $12 \mu g$ of reference RNA (Stratagene) were Cy5-labeled. All other steps, including hybridization, were performed following the protocol published by P.Brown et al. (see http://cmgm.stanford.edu/pbrown for details) except that a PCR-purification kit (Qiagen, Hilden, Germany) was used for cDNA purification. (For a detailed protocol of hybridization see www.genomics.uni-freiburg.de)

BrdU-test

BrdU-(5-Brom-2-desoxyuridin)-tests were purchased from Roche (Grenzach-Wyhlen, Germany) and done according to the manufacturer's specification simultaneously using the same cells, same drug dilutions and identical culture conditions.

Preparation and storage of drugs

CsA and EVL were kindly provided by Novartis Pharma (Basel, Switzerland), FK by Astellas Pharmaceuticals (Munich, Germany) and SRL by Wyeth (Münster, Germany), mycophenolic acid and p-PHA were purchased from Sigma (Munich, Germany).

Drugs were kept lyophilized at -20° C until preparation of stocks at a concentration of 1 mg/ml for SRL and 10 mg/ml for all others. Stocks were prepared in ethanol containing 20% of tween 20 for CsA, FK, SRL, and EVL and in methanol for MMF and also kept at -20° C until dilution in medium. The first step was done in pure medium, all following steps in medium containing fetal calf serum.

Drug concentrations were chosen from *in vitro* data published previously.⁽¹¹⁻¹³⁾

Microarrays

cDNA microarrays were produced and processed essentially according to the Stanford protocol described by Eisen and Brown 1999.⁽¹⁴⁾ Approximately 7,700 annotated genes from the RZPD (Resource Center and Primary Database, Berlin, Germany) including 1152 clones from the so-called lymphochip (https://www.rzpd.de) were obtained as bacterial stocks. Plasmids were purified using the Qiagen 96-well Turbo Kit (Qiagen, Hilden, Germany), and inserts were purified by PCR using vector primers flanking the individual inserts (5'-CTG CAA GGC GAT TAA GTT GGG TAA C-3' and 5'-GTG AGC GGA TAA CAA TTT CAC ACA GGA AAC AGC-3').

PCR products were purified by ethanol precipitation and resuspended in ddH2O. Aliquots were transferred into 384-well plates, dried and resuspended in 3 × SSC/1.5M betain to a final concentration of approximately 40 ng/µl. Printing was performed on aminosilane coated slides (CMT-GAP II Slides, Corning, NY, USA), using an arrayer that was assembled according to specifications by the Stanford group using software provided by Joe de Risi (http://cmgm.stanford.edu/pbrown).

Semiquantative RT-PCR

Total RNA $(1 \mu g)$ from all samples of one donor was purified with the RNase free DNase set (Qiagen, Hilden, Germany) and reversely transcribed into cDNAs using oligo (dT) 12-18 primer and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Each singlestranded cDNA was diluted for subsequent PCR amplification. PCR was carried out with GAPDH as an internal control in each reaction vial. The following primers were used (5'-3'): GAPDH: TGG AAA TCC CAT CAC CAT CT and GTC TTC TGG GTG GCA GTG AT; E4BP4 (X64318): GAA CCC TCG ATG GTG TCA AG and TCT TGG CTC CCT TGT GTA GC; Fructose Bisphosphatase (U21931): GCC GTG TTA GAC GTC ATT CC and TAA GGT GCA CAG CAG GTC AG; MCP 2 (Y16645): AGC CAC TTT CAG CCC TCA G and AAT CCC TGA CCC ATC TCT CC; IL-2: GCA ACT CCT GTC TTG CAT TG and CAG TTC TGT GGC CTT CTT GG.

To determine the optimal number of PCR cycles for all primer pairs a set of preliminary tests was performed using RNA of one donor. An initial denaturation at 94°C for 5 min followed by 28 cycles of denaturation for 30 sec, annealing for 1 min at 55°C and elongation for 2 min at 72°C was found to give best results in all reactions. Products were run on 3% agarose gels and bands revealed using ethidium bromide staining. Bands were evaluated in relation to the corresponding GAPDH-band using the Scion Imaging software (Scion, Frederick, MD, USA).

Data analysis

Signal intensities were measured by an Axon 4000A scanner using GenePix 3.0 software (Axon Instruments Inc., Union City, CA, USA). Artifacts, if not detected by the software, were excluded manually. Image and data files, array layout, as well as all relevant information according to the MIAME guidelines (Minimum Information About a Microarray Experiment⁽¹⁵⁾) were transferred into the GeneTrafficDuo database (Microarray Data Management and Analysis Software, Iobion Informatics, LLC, La Jolla, CA, USA).

To exclude artifacts near background range, all spots were eliminated when sample intensity or reference intensity was less than 50 above the local background. Local background was subtracted from spot intensities. Normalization was performed with the Lowess (Locally weighted scatter plot smoother) sub-grid normalization method. Sub-grid normalization calculates the normalization factor for each of the 16 sub-grids independently and therefore is, compared to global normalization, relatively insensitive to local variations on the array.⁽¹⁶⁾

For statistical analysis of differentially expressed genes we fit a linear model to the gene expression data *X* to estimate the influence α_p of the patients and the effect β_d of the drugs using the following formula:

$$\begin{split} X_{pdi} = & \alpha_p + \beta_d + \varepsilon_{pdi} \\ p = 1, \dots, npatient, d = 1, \dots, ndrugs, i = 1, \dots nreplicates. \end{split}$$

p enumerates the patients treated with drug d and $\varepsilon_{\rm pdi}$ is Gaussian noise. The index i count the number of replicates.

Using this model we could eliminate patient specific effects on gene expression data. In the case of n=1 this corresponds exactly to a paired t-Test.

For each gene, the fit of the linear model provides a probability that the applied drug has a vanishing effect. To adjust these obtained *p*-values, the method by Benjamini⁽¹⁷⁾ was applied to control for multiple testing (FDR = false discovery rate). For each drug 50 genes with the most significant effect (smallest pFDR = p-value adjusted for multiple testing) were selected and agglomerative hierarchical clustering introduced by Kaufman⁽¹⁸⁾ was performed using the R statistical software package (www.r-project.org).

In order to screen on synergistic, possibly supra-additive effects, genes were selected which fulfilled the following three criteria: First, genes regulated either up or down less than 1.5-fold (+/- log 0.585) in the drug combination were eliminated. Second, pFDR for the change of expression level in the drug combination versus controls had to be <0.05. Third, regulation of combination versus each single substance and of combination versus expected fold-regulation (calculated as sum of effects of single substances) had to be at least 1.5-fold.

Results

One goal of this study was to measure the effects of immunosuppressants in various clinically relevant combinations on the gene expression profile of human PBL. We used the accepted model⁽¹¹⁾ of culturing PHA treated PBL in the presence of immunosuppressants.

Effects of immunosuppressants on cell proliferation and IL-2 production

We first tested the effect of each single substance on lymphocytic proliferation. Only MMF and none of the other single drugs in the chosen concentrations had an effect on lymphocyte proliferation. In combination of calcineurininhibitors with TOR-inhibitors there was a tendency to decrease proliferation in all cases (Figure 1a).

Semiquantitative RT-PCR was used to measure IL-2 expression. As expected and reported before⁽¹¹⁾ IL-2production of PHA stimulated lymphocytes was reduced in presence of calcineurin-inhibitors and EVL and SRL. Combination of those drugs seemed to further reduce IL-2 production in all cases (Figure 1b). Mycophenolic acid did not affect the expression level of IL-2 in our system.

Effects of solvents and immunosuppressants on gene expression

The three different solvents constitute three different types of negative controls. Initially, we tried to ensure that



Figure 1. a: BrdU-Test was performed with each series of cell cultures. Pooled results of BrdU incorporation shown as percentage of control ± SD are given in figure 1a. b: Semi-quantitative RT-PCR using primers for IL-2 and GAPDH were performed on RNA from PHA stimulated PBL after incubation in presence of different immunosuppressive drugs. Expression level of GAPDH was used for internal normalisation. Expression levels of IL-2 were plotted as percentage of control (PHA only, no drugs).

the solvents did not lead to significant changes in gene expression measurements. No significant difference in gene expression was found by a t-test between the solvents after correction for multiple testing. Actually, all pFDR values were found to be close to one. The variability of the measurements within the negative control subset of the arrays (median SD=0.227) undercut the variability in the immunosuppressant samples (median SD=0.325) by a factor of 1.43. Figure 2 shows this difference in the measured variability. Hence, drug related changes in expression were estimated as fold induction in comparison to all three solvents as a common negative reference group.

Reproducibility of array data

On our array there were several pairs of genes spotted twice with different cDNA-fragments. The results obtained from those duplicates were highly consistent (Figure 3) and emphasize the high reproducibility of the array data.

As only one concentration of each drug was tested the experiment was not laid out to detect differences between drugs belonging to the same class. In fact, comparison between the calcineurin-inhibitors (CsA versus FK) and the TOR-inhibitors (EVL versus SRL) yielded very little significant (pFDR<0.05) differences in gene expression. Comparing CsA and FK 63 significantly regulated genes were found. However, only for one gene the difference of expression exceeded the 1.5-fold (AB020644). The TOR-inhibitors differed significantly in 47 genes, again a difference>1.5-fold was found for 2 genes only.

Agglomerative clustering

Agglomerative hierarchical clustering using all 7700 genes separated the samples mostly in a donor specific way (Figure 4a). Focusing on changes of gene expression of treated PBL versus untreated controls from the respective donor, however, an expression pattern emerged by which the samples could be grouped according to treatment. The regulation of the genes involved in these drug specific patterns seems to be specific for the treatment condition and suggests the



Figure 2. Frequency distribution of measurement variability. A clear decrease in standard deviation is observed within the negative controls (black) in comparison to the standard deviation within the treated subset (grey) of microarrays.



Figure 3. Histogram of expression levels determined by microarray with two different c-DNA fragments from the c-MAF gene from three donors including SD. The histogram shows the corresponding means of expression levels from microarray data of three donors (after sub-traction of control) on a log 2 scale including SD.

presence of a transcriptional fingerprint (Figure 4b). We conclude that inter-individual variability can be overcome by deducting gene- and donor-specific control values for each gene. All further analyses to identify and describe "transcriptional fingerprints" or single "genes of interest" were done on "fold-changes" in gene expression compared to control (PHA stimulated PBLs) and their respective pFDR-values.

Group-specific effects of the immunosuppressants

Looking for group-specific effects of immunosuppressants genes were selected which were significantly regulated in the presence of one class of drugs, but not by other drugs. Calcineurin-inhibitors and TOR-inhibitors seemed largely to affect regulation of the same genes. However, still some genes could be identified which were regulated in a drug-specific way, for instance several monocyte chemoattractants (e.g., MCP-2 shown in Figure 5) were markedly down-regulated by TOR-inhibitors, but not affected in their regulation by calcineurin-inhibitors.

Additive effects of drug combinations

From the known biological properties of the drugs we expected that certain genes would be regulated in a synergistic ("supra-additive") fashion. To find such genes we selected for 1. significant regulation of the combination versus control and 2. changes of geneexpression of the combination exceeding the 1.5-fold (log 0.585) compared to control, either single substance or the sum of effects of the single substances. 309 genes fulfilled these criteria in at least one drug combination, calcineurin-inhibitors and MMF for 34 genes, calcineurin-inhibitor and TOR-inhibitor for 284 genes (including 9 genes which were selected in both kinds of combinations). We focused our further evaluation of supra-additive effects on the latter combinations, as the genes selected by our criteria were much more frequent.

Theoretically, such genes should be regulated in a similar way in every combination of a calcineurin-inhibitor with a TOR-inhibitor (CsA-EVL, CsA-SRL, FK-EVL, FK-SRL). As expected, 110 of those genes fulfilled our selection criteria in more than one tested combination and 55 genes in least at 3 of 4 (A list of those 55 genes is shown in Table 1, note that it contains three of the duplicate pairs mentioned above and shown in Figure 3). Re-examination of this selection based on means and p-values calculated for the entire substance group confirmed that gene expression levels between single substance (TOR-inhibitor and calcineurin-inhibitor respectively) and combination differed significantly in all cases.



Mycophenolic acid

Figure 4. a: Hierarchical clustering based on Euclidean distance was done using all genes and all samples. The branches are characterised by origin from a common donor (color), modality of treatment (shapes) has a minor influence on the clustering. b: Hierarchical clustering based on Euclidean distance was done using the 50 genes with smallest pFDR-values calculated for drug or drug combination effects by fitting a linear model as described in Methods. Using this approach the modality of treatment (shapes) becomes the major determinant for the sorting of samples. The dendrogram's two major branches divide samples incubated with combination of calcineurin-inhibitor and TOR-inhibitor, from samples treated with single substances. The latter is subdivided in a branch with MMF samples, one with TOR-inhibitor samples and a third with calcineurin-inhibitor treatment, one sample stands apart. Note that one set of duplicates for FK-MMF and two sets for MMF for donor three were combined in Figure 4b.

Approximately two thirds of these genes were upregulated, one third was down-regulated. Functionally many of the up-regulated genes are involved in inflammation and innate immune response (e.g., fibroblast collagenase inhibitor, type IV collagenase, alpha-1 -antitrypsin-related protein), whereas many of the down-regulated genes are directly involved in the cell cycle, CTL (cytotoxic T-lymphocyte) function (such as granzyme B), apoptosis (bcl-xL), signaling (E4BP4) or transcription (c-MAF).

As shown in Figure 5 for 3 genes, microarray data matched the results of semiquantitative RT-PCR well.

Discussion

Long term immunosuppression after transplantation relies on the beneficial effects of immunosuppressive combination therapy. However, biological mechanisms of drug synergy are incompletely understood and an analytic basis for development of individually "tailored" immunosuppression is desirable.

With a systematic approach of collection of gene expression profiles Lamb et al.⁽⁹⁾ created a resource to discover functional connections of actions of drugs from gene expression data of *in vitro* experiments. Hence, patterns of transcriptional response to immunosuppression most likely also indicate drug effects or possible adverse effects more precisely than single markers.

With our microarray experiments we could show that immunosuppressive drugs generate a distinctive gene expression pattern in an *in vitro* setting in only 24 h. By use of multiple biological controls we could reduce interindividual variance. Cluster analysis revealed a unique regulation in samples treated with combination immunosuppressive therapy. Genes with a strikingly supra-additive regulation under combination of calcineurin-inhibitors and TOR-inhibitors were extracted by a mathematical model. In many cases genes involved

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Figure 5. Verification of expression of candidate genes by semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR). GAPDH (upper band) and candidate gene (lower band) were amplified simultaneously from cDNA from one donor. Above histogram shows the corresponding means of expression levels from microarray data of three donors (after subtraction of control) on a log 2 scale including SD. The samples are in the following order: CsA, FK, MMF, SRL, EVL, blank, CsA+MMF, CsA+SRL, CsA+EVL, blank, FK+MMF, FK+SRL, FK+EVL. 254×x190 mm.

in inflammation and innate immune response were up-regulated, whereas chemokines and genes involved in cell cycle or specific CTL function where downregulated.

Distinctive patterns of gene expression induced by genetic alteration or external influences have been called "transcriptional fingerprints." They can be defined through cDNA microarrays by simultaneously screening thousands of genes in a non-hypothesis driven way. Microarray studies have already been used successfully *in vivo* and *in vitro* to identify such "fingerprints" of transplant rejection and lymphocyte activation.^(7,19)

Immunosuppression works by influencing mechanisms of activation of lymphocytes and monocytes. The evaluation and prediction of drug effects *in vivo* is a difficult task. Large inter-individual differences in gene expression levels, different medical conditions or variable co-medications would render variance unacceptably high. An individually useable *in vitro* test system which could eventually be correlated with clinical outcome data appears to be a valuable tool. We chose an easily reproducible *in vitro* cell culture assay of lymphocyte activation with standardized conditions to test whether "transcriptional fingerprints" are generated by treatment with immunosuppressants. We are aware of the limitations of *in vitro* assays in predicting biological processes in patients. Yet, Dudley et al. could successfully use an *in vitro* model of cyclosporine response in the prediction of individual response to immunosuppression.⁽²⁰⁾

Another possible drawback to gene expression analysis is that a pattern described on a post-transcriptional level may be substantially distorted post-translationally. However, in a study of *in vitro* activated T-cells treated with and without calcineurin-inhibitors, microarray analysis proved to be a valid tool to identify novel transcriptionally regulated genes.⁽²¹⁾ Results were confirmed on protein level and new therapeutic targets could be identified.

Using cut-off-values for minimal, presumably "relevant" regulation and p-value criteria to eliminate data of unclear significance we identified clear cut expression patterns for many genes. We realize that

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Table 1. List of genes with supra-additive regulation by combination of calcineurin-inhibitors and TOR-inhibitors.

ID Name Category Cit Tit CH D83200 Ficoln-1 Complex functions lic.ell cycls 5.5 5.8 3.23 Zäsä1 Fibrinogen-like protein (T+19) Unknown function in immune response/Inflammation 1.01 0.73 2.58 U04988 Riboundcawe K procursor Immune response/Inflammation 1.58 -0.31 2.33 N01683 alpha 1-antitrypsin Immune response/Inflammation 0.71 0.83 2.33 N01683 Neuroendocrine-specific protein A Vescilar trafficing 0.83 0.41 2.16 N01683 Inplat - antitrypsin Fercecptor 0.33 0.41 2.16 N023011 Neutrophil ackase factor (p67-phon) Redux regulation 0.83 0.60 1.77 N03883 c fres proto-oncogene cytokine receptor 0.33 0.60 1.77 N04884 Alpha-1 antitrypsin-related protein Immune response/ Inflammation 0.49 0.45 1.77 N02884 Christon-reducible cytochrome P450 Complet functions Immune re	Table 1. List of genes	with supra-additive regulation by combination	If of calcineurin-minorors and ron-minoror	3.		
DB4390Fieldin-1Complex functions ind. cell cycle1.30.383.23Z36511Fibrioblast collagenase inhibitorImmune response/Inflammation1.010.792.58N25218Fibrioblast collagenase inhibitorImmune response/Inflammation-0.111.202.48X62466CAMPATH-1 (CD62)Unknown function in immune response1.880.762.41X01633alpha 1-antitrypsinImmune response/Inflammation1.580.312.53X01633Nauroendoctne-specific protein AVesicular trafficking0.630.502.19X02573Fc receptor TC9Fc receptor0.350.642.19X02634Interlevalin-13 receptorCarbohydrate metabolism0.870.062.63V12131Fnictose-1, 6-biphophataseCarbohydrate metabolism0.530.602.63V203684Interlevalin-13 receptorcytokine receptor0.750.021.87V03683C-fms proto-oncogenecytokine receptor0.750.021.87V03684Diorin-inducible cytochrome P450Complex function in immune response0.490.451.77V03684Diorin-inducible cytochrome P450Complex function in immune response0.490.451.77V03684CD36CD36Complex function in immune response0.490.451.77V03684Otorin-inducible cytochrome P450Complex function in immune response0.490.451.72V03684CD36CD36Compl	ID	Name	Category	CI	TI	CI+TI
Z3651 Phrinogen-like protein (p149) Unknown function in imunue response 1.30 0.84 3.21 NS218 Phrinobas collagenases inhibitor Immune response/Inflammation 1.01 0.76 2.48 NS4866 CAMPATF1 (CDw32) Unknown function in imunue response/Inflammation 0.76 2.41 NS1638 alpha I- antitrypsin Immune response/Inflammation 0.71 0.53 2.23 NS1638 alpha I- antitrypsin Immune response/Inflammation 0.61 0.50 2.19 NS2573 Pe receptor TC3 Neuroendoortine-specific protein A Vesicular tradibian 0.66 0.50 2.65 V121931 Proteo-oncogene Cytokine receptor 0.33 0.60 1.71 N05868 Console-functions 0.48 0.53 1.77 T5154 Neuropilin 1 angiogenesis, regulatory T cells 0.75 0.60 1.71 N05868 C336 C5 2.91 1.71 1.71 N05868 C336 C5 2.60 1.45 1.77	D83920	Ficolin-1	Complex functions incl. cell cycle	1.55	0.38	3.23
N25218 Fibroblast collagenase inhibitor Immune response/Inflammation 1.01 0.79 2.58 V62466 CAMPATF-1 (CMx32) Unknown function in immune response/Inflammation 1.68 -0.31 2.33 N01633 alpha I-antitryspin Immune response/Inflammation 0.71 0.53 2.25 100331 Neuroendocrine-specific protein A Vesicular trafficking 0.83 0.60 2.51 V10333 Neuroendocrine-specific protein A Vesicular trafficking 0.83 0.60 2.52 V10333 Neuroendocrine-specific (p67-phox) Redox regulation 0.87 0.02 1.87 V103684 Alpha-1-antitrysin-claid optotin Immune response/Inflammation 0.43 0.77 V.00 1.73 V103684 Diodni-inducible cytochrome P450 Complex functions 0.48 0.45 1.77 V103680 Diodni-inducible cytochrome P450 Complex functions 0.48 0.45 1.77 V103680 Diodni-inducible cytochrome P450 Complex functions 0.48 0.45 1.77 V103680 </td <td>Z36531</td> <td>Fibrinogen-like protein (pT49)</td> <td>Unknown function in immune response</td> <td>1.13</td> <td>0.84</td> <td>3.21</td>	Z36531	Fibrinogen-like protein (pT49)	Unknown function in immune response	1.13	0.84	3.21
U6999 Ribonuclease & precursor Transcription 1.1 1.20 2.48 N62206 CAMPATFH (CDWS2) Unknown function in immune response/Inflammation 0.88 0.56 2.33 N05633 alpha I- antitrypsin Immune response/Inflammation 0.71 0.53 2.25 L10333 Neuroendocrine-specific protein A Vesicular trafficking 0.63 0.50 2.19 N02573 Fe receptor TC9 Fe receptor 0.88 0.56 2.19 N20211 Neurosphilo scidase factor (p67-phox) Redox regulation 0.88 0.66 2.16 N128888 Interlevenkin-13 receptor cytokine receptor 0.73 0.60 1.77 N03683 e-fms proto-oncogene Unknown function in immune response 0.48 0.45 1.77 N03683 cofms proto-oncogene Unknown functions 0.48 0.50 1.71 N03683 cofms proto-oncogene Unknown functions 0.48 0.53 1.71 N03683 CD36 Linkin veresponse/ Inflammation 0.43 0.53	N25218	Fibroblast collagenase inhibitor	Immune response/Inflammation	1.01	0.79	2.58
X82466 CAMPATH-1 (CDwS2) Unknown function in immure response/Inflammation 1.58 -0.76 2.41 D06070 Type IV collagenase Immure response/Inflammation 1.58 -0.51 2.32 X01683 alpha 1-antitryspin Immure response/Inflammation 0.63 0.62 2.25 X02573 Fc receptor TCS Fc-receptor 0.93 0.41 2.16 X032011 Neuroodnocrine-specific protein A Pecreceptor 0.93 0.41 2.16 X03011 Neuroofni code for for phoxi Redox regulation 0.87 0.62 2.02 M19804 Alpha - Lantitryspin - reductal protein Immure responses/Inflammation 0.33 0.67 1.87 X03683 c-fms proto-oncogene cytokine receptor 0.49 0.45 1.77 W30848 Dioxin-inducible cytochrome P450 Complex functions 0.49 0.45 1.77 W30849 Dioxin-inducible cytochrome P450 Complex functions 0.49 0.45 1.77 W30849 Dioxin-inducible cytochrome P450 Complex functions <td>U64998</td> <td>Ribonuclease k6 precursor</td> <td>Transcription</td> <td>-0.11</td> <td>1.20</td> <td>2.48</td>	U64998	Ribonuclease k6 precursor	Transcription	-0.11	1.20	2.48
196070Type IV collagenaseImmune response/Inflammation1.580.532.25L10333alpha 1-nnitrypsinImmune response/Inflammation0.630.502.18X82573F receptor TC9Fe-receptor Inflicking0.630.642.16X82573F receptor TC9Carbolyfrate metabolism0.850.252.09M32011Neutrophil oxidase factor (p67-phox)Redox regulation0.630.602.05V82585Interleukin-13 receptor0.530.601.49V83663c-fms proto-oncogenecytokin receptor0.530.601.47V83683Dioxin-inducible cytochrome P450Complex functions in immune response0.480.451.77V03686Dioxin-inducible cytochrome P450Complex functions in immune response0.480.451.77V03686Dioxin-inducible cytochrome P450Complex functions in immune response0.480.451.77V03686Dioxin-inducible cytochrome P450Complex functions0.490.531.72V03686Dioxin-inducible cytochrome P450Complex functions0.490.531.72V03686Dioxin-inducible cytochrome P450Complex functions0.490.531.72V03786Dioxin-inducible cytochrome P450Complex functions0.410.421.62V17213muse response/infammation0.470.681.631.64V12324Divocyte antigen CD1cAntigen presentation0.410.531.63 <tr< td=""><td>X62466</td><td>CAMPATH-1 (CDw52)</td><td>Unknown function in immune response</td><td>0.89</td><td>0.76</td><td>2.41</td></tr<>	X62466	CAMPATH-1 (CDw52)	Unknown function in immune response	0.89	0.76	2.41
X01683alpha 1-antirypsinImmune response/Inflammation0.710.532.25X10333Neuroendocrine-specific protein AVesicular trafficking0.430.412.16X12373Frecceptor TC9Carbolydrate metabolism0.850.252.09V21311Neutoreh-16-biphosphataseCarbolydrate metabolism0.350.762.02U62856Interleukin-13 receptorcytokine receptor0.550.762.02V19864Alpha1-antitrypsin-related proteinImmune response/ Inflammation0.430.451.77T83429similar to CD9Unknown function in immune response0.490.451.77V103888Dioxin-inducible cytochrome P450Comples functions0.490.451.71V10388Dioxin-inducible cytochrome P450Comples functions0.490.451.71V103888CD36CD3Internetion0.440.021.53V107902T1s1dRNA binding protein0.440.021.43V1233Meuropilin 1R04N.021.441.42V1234Unsula 5' feal A-gamma-globinCel cycle0.640.011.40V1234MaboguninDid differentiation0.410.221.43V1234MaboguninDid differentiation0.420.291.33V1235Fedil mENA for 1g forecpinceProteponse/ Inflammation0.420.411.27V1234MaboguninDid for cecptor0.410.421.27 </td <td>J05070</td> <td>Type IV collagenase</td> <td>Immune response/Inflammation</td> <td>1.58</td> <td>-0.31</td> <td>2.33</td>	J05070	Type IV collagenase	Immune response/Inflammation	1.58	-0.31	2.33
L1033 Neuroendocrine-specific protein A Vesiclar trafficking 0.63 0.50 2.16 V22573 F creceptor TC9 0.63 0.41 2.16 V21301 Fructose-1.6-biphosphatase Carbohydrate metabolism 0.85 0.25 2.09 M32011 Neutophil oxidase factor (p57-phox) Redox regulation 0.87 0.68 2.05 M19684 Alpha-1-antitypsin-related protein immune response/ Inflammation 0.53 0.60 1.43 T33429 similar to CD9 Unknown function in immune response 0.49 0.45 1.77 T03584 Dioxin-inducible cytochrome P450 Complex functions 0.49 0.43 1.71 M88298 CD36 Tist 14 Raman-globin Cell cycle 0.46 0.02 1.50 M28291 Unsual 5'-fetal Agama-globin Cell cycle 0.36 0.50 1.45 M28292 Unsual 5'-fetal Agama-globin Cell cycle 0.31 0.33 1.40 M28294 Unsual 5'-fetal Agama-globin Cell cycle 0.34 <t< td=""><td>X01683</td><td>alpha 1-antitrypsin</td><td>Immune response/Inflammation</td><td>0.71</td><td>0.53</td><td>2.25</td></t<>	X01683	alpha 1-antitrypsin	Immune response/Inflammation	0.71	0.53	2.25
XE2573Fc receptor TC9Fc receptor0.330.412.16VI21931Fructose-1-6-biphosphataseCarbolytrate metabolism0.470.082.02M32011Neutrophil oxdase factor (p67-phox)Rodox regulation0.350.762.02VI8208AInterleukin-1 arceeptorcytokine receptor0.350.760.201.34X036GA-fms proto-mocogenecytokine receptor0.750.421.77V03688Dioxin-inducble cytochrome P450Complex function immune response0.490.451.77V03688Dioxin-inducble cytochrome P450Complex functions0.490.451.77V03688Dioxin-inducble cytochrome P450Complex functions0.440.021.51V07002Tis11dAnty sepsonalin proteinse0.440.021.46M32249Umusula 5'-fetal A-gamma-globinCell cycle0.360.091.46M32249Umusula 5'-fetal A-gamma-globinCell cycle0.360.021.39V71213mGST-1Rodox regulation0.240.201.39N71535FcRII mRNA for Ig G receptorCytokine receptor0.450.191.37M42210Cathegrin function immune response / Inflammation0.240.201.39N71535FcRII mRNA for Ig G receptorCytokine receptor0.450.141.27M42210Cathegrin function immune response / Inflammation0.240.201.39N71535FcRII mRNA for Ig G receptor	L10333	Neuroendocrine-specific protein A	Vesicular trafficking	0.63	0.50	2.19
U21931Fructose-1.6-biphosphataseCarbohydrate metabolism0.850.252.09M23011Neutrophl oxidase factor (p67-phox)Redox regulation0.350.672.05U62858Interleukin-13 receptorcytokine receptor0.350.601.34M19684Alpha-1-antitrypsin-related proteinImmune response/ Inflammation0.530.601.37T83429similar to CD9Unknown function in immune response0.490.451.77T31594Neuropilinangiogenesis. regulatory T cells0.750.001.73U03688CD36Thrombospondin receptor0.440.021.64M88398CD36Thrombospondin receptor0.310.081.45U71213mCGT-1Redox regulation0.270.081.45M28296Interleukin-Treceptor (IL-7)Cytokine receptor0.310.031.40M29696Interleukin-Treceptor (IL-7)Cytokine receptor0.410.221.33J03809g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.720.091.33J03819g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.120.301.72J0364Hordy-g-size XiganAntigen presentation0.120.321.37J03899g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.120.331.37J0553TIM-2Antigen presentation0.120.341.32J05543 <td>X62573</td> <td>Fc receptor TC9</td> <td>Fc-receptor</td> <td>0.93</td> <td>0.41</td> <td>2.16</td>	X62573	Fc receptor TC9	Fc-receptor	0.93	0.41	2.16
Ma2011Neutrophil oxidase factor (p67-phox)Redox regulation0.870.062.05M19684Alpha-1-antitrypsin-related proteinImmune response/ Inflammation0.530.601.94X03663c-fms proto-oncogenecytokine receptor0.750.021.87W138242similar to CD9Unknown function in immune response0.490.451.77W03688Dioxin-inducible cytochrome P450Complex functions0.490.451.77W03704Ts11dRNA binding protein0.490.531.61W03704Ts11dRNA binding protein0.470.081.73W88895CD36CB4Mation receptor0.440.021.50W28827Unusual 5'-fetal A spanma-globinCell cycle0.360.501.45W29696Interlexikn-7receptor (IL-7)Cytokin receptor0.410.221.33U71213mGST-1Kedox regulation0.240.091.33J03909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.240.091.33J0393FBI ImRNA for JG creceptorFe-receptor0.010.051.33J0533TBI Y-2Immune response/ Inflammation0.120.030.12J0534HIMPA Org JG creceptorFe-receptor0.010.051.33J05393TBI Y-2Immune response/ Inflammation0.010.051.13J0533TBI Y-2Matiogen presentation0.020.041.2	U21931	Fructose-1_6-biphosphatase	Carbohydrate metabolism	0.85	0.25	2.09
U62836Interleukin-13 receptorcytokine receptor0.350.672.02N19684Alpia-1-antirypsin-related proteinImmune response/ Inflammation0.550.021.87N23663c-fms proto-oncogenecytokine receptor0.750.021.77TB3420similar to CD9Complex functions i mimune response0.490.451.77TB1594Neuropilin 1angiogenesis, regulatory T cells0.750.001.73V03808CD36Thrombospondin receptor0.440.021.61M28249Unusal 5'- fetal A-gamma-globinCell cycle0.130.031.40M2249Unusal 5'- fetal A-gamma-globinCell cycle0.130.031.40M28496Interleukin-7 receptor (IL-7)Cytokine receptor0.310.031.40M28496Interleukin-7 receptor (IL-7)Cytokine receptor0.130.031.30N71535FeRI ImRNA for Ig G receptorAntigen presentation0.120.031.37N72535FeRI ImRNA for Ig G receptor0.161.331.31N7535ThMP-2Immune response/ Inflammation0.120.041.17N4623764TMNcoyte antigen CDIcAntigen presentation0.120.041.17N452376TMMCof95621134Na nonciton yet0.060.161.33N7533TMM-2Immune response/ Inflammation0.120.041.17N452376TMMCof95621134Na nonciton yet0.060.161.33<	M32011	Neutrophil oxidase factor (p67-phox)	Redox regulation	0.87	0.06	2.05
M1964Alpha 1-anitrypsin-related proteinImmune response/ Inflammation0.530.601.74X03663c-fms proto-oncogeneVirknown function in immune response0.490.451.77TB3429similar to CD9Complex functions0.490.451.77V03683Dioxin-inducible cytochrome P450Complex functions0.490.531.71V07802Tis11dangloenesis. regulatory T cells0.490.531.71V07802Tis11dRNA binding protein0.490.531.51M28827Tymocyte antigen CD1cAtige presentation0.410.221.45M28287Unusual 5'- fetal A-gamma-globinCell cycle0.660.660.661.45M28969Interleduk-7 receptor (IL-7)Cyrokine receptor0.410.221.31J03090g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.420.201.33J03091g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.410.121.33J14221Cathepsin B proteinaseAntigen presentation0.420.401.16J05533InJ 3Viscual trafficking0.011.171.16J05693C21134INAGp958C21134No annotation yet0.010.111.16J05693CLipdong peroxidaseRedox regulation0.430.411.22J04033Gibtarthine peroxidaseRedox regulation0.450.160.93J05621Histone H	U62858	Interleukin-13 receptor	cytokine receptor	0.35	0.76	2.02
X03663c-fms proto-oncogenecytokine receptor0.730.621.177T83429similar to CD9Unknown function inmune respons0.490.451.77T83454Neuropilin 1angiogenesis, regulatory T cells0.750.001.73U07602TIs11dRNA binding protein0.490.331.71M98398CD36Thrombospondin receptor0.440.021.50M2827Thymocyte antigen CD1cAntigen presentation0.270.081.46M32249Unusual 5'- fetal A-gamma-globinCleycle0.310.331.41M3249Instreteukin- Treceptor (II-7)Cytokine receptor0.310.331.41M3249Instreteukin- Treceptor (II-7)Cytokine receptor0.340.341.43M3909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.220.391.37M15235FeRI mKNA for Ig G receptorAntigen presentation0.440.401.37M1623744Tymocyte antigen CD1cAntigen presentation0.430.141.227M14221Cathepsin B proteinaseAntigen presentation0.430.141.127M5593rab 1.3IMAGp9582C1134IMAGp9582C1134No annotation yet0.010.001.61M6591Histome H1Woing functions in immune respons/0.030.910.911.91M1421Gatepsin EproteinaseComplex functions in immune respons0.030.911.91M152	M19684	Alpha-1-antitrypsin-related protein	Immune response/ Inflammation	0.53	0.60	1.94
TB3429similar to CD9Unknown function in immune response0.490.451.77U03688Dioxin-inducible cytochrome P450Complex functions ir munue response0.490.451.77TIS194Neuropilin 1angiogenesis. regulatory T cells0.490.531.71U07802Tis1 IdRNA binding protein0.490.531.71M88398CD56Thromospondin receptor0.440.021.561M2827Thymocyte antigen CD1cAntigen presentation0.270.081.45M32249Unsual 5' fetal Agamma globinCell cycle0.310.031.41M29596Interleukin-7 receptor (IL-7)Cytokine receptor0.310.031.31J03090ginterferon-inducible protein (IP-30)Immune response/Inflammation0.240.201.37J1623784RHMPorg ta greeceptorAntigen presentation0.440.161.37J05533Thymocyte antigen CD1cAntigen presentation0.340.141.27U190540Thymocyte antigen CD1cAntigen presentation0.340.141.27U190543Thymocyte antigen CD1cUnknown function in immune response0.100.161.31U19553Tel AgestraftaNage presentation0.340.141.27U19544Thymocyte antigen CD1cUnknown function in immune response0.011.75U19553Tel AgestraftaNage presentation0.340.141.27U195642U134Natogen p	X03663	c-fms proto-oncogene	cytokine receptor	0.75	0.02	1.87
U0388Dixin-inducible cytochrome P450Complex functions0.490.451.71T51594Neuropilin 1angiogenesis.regulatory Tcells0.691.73U07002Tis11dRNA binding protein0.490.021.50M8389CD36Thrombospondin receptor0.490.021.45M82827Thymocyte antigen CD1cAntigen presentation0.270.081.46M2249Unusual 5- fetal A-gamma-globinCell cycle0.360.311.43M29696Interleukin-7 receptor (IL-7)Cytokine receptor0.310.021.37J03909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.240.091.37J03930g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.400.161.33J05533TMP-2Immune response/ Inflammation0.400.161.32J05534TMP-2Immune response/ Inflammation0.410.161.32J0553TMP-2Immune response/ Inflammation0.410.161.32J0553TMP-2Immune response/ Inflammation0.430.411.32J0553TMP-2Immune response/ Inflammation0.410.161.32J0553TMP-2Immune response/ Inflammation0.410.160.16J0564TMP-2Immune response/ Inflammation0.410.160.41J0553TMP-2Immune response/ Inflammation0.410.411.52 <t< td=""><td>T83429</td><td>similar to CD9</td><td>Unknown function in immune response</td><td>0.49</td><td>0.45</td><td>1.77</td></t<>	T83429	similar to CD9	Unknown function in immune response	0.49	0.45	1.77
T51594Neuroplin 1angiogenesis regulatory T cells0,750,001,73107802Tis11dRNA binding protein0,490,531,71M98398DD36Thrombospontin receptor0,440,221,81M32249Unusual 5' fetal Agamma-globinCell cycle0,310,331,46M32249Unusual 5' fetal Agamma-globinCell cycle0,310,331,40M28966Interlexin-7 receptor (IL-7)Cytokine receptor0,310,331,40AB01116MahoguninTh2 differentiation0,720,901,33N71335FCRII mRNA for Ig G receptorFc-receptor0,450,191,33N71355FCRII mRNA for Ig G receptorAntigen presentation0,120,301,17M4221Cathepsin B proteinaseAntigen presentation0,120,301,17M1421MAfop958C21134No annotation yet0,010,021,18N75593na 13Vosicular trafficking0,010,001,17IMAGp958C21134IMAGp958C21134No annotation yet0,010,001,17IMAGp958C21134Idsone H1Histone0,060,220,93V09433Glutathione peroxidaseRedox regulation0,020,030,91V12959Histone H1Histone0,060,230,91V36592Ribomuclease 6 precursorTranscription infimmure response0,030,300,30V1295Ribomuclease 6 precursor	U03688	Dioxin-inducible cytochrome P450	Complex functions	0.49	0.45	1.77
U07020Tis11dRNA binding protein0.490.531.71M98398CD36Thrombospondin receptor0.440.021.50M2827Thromocyte antigen CD1cAntigen presentation0.270.081.46M32249Unusual 5'-fetal A-gamma-globinCell cycle0.360.031.43M29696Interleukin-7 receptor (IL-7)Cytokine receptor0.310.031.40A8011116MahoguninTh2 differentiation0.240.021.39J03909g-interferon-inducible protein (IP-30)Immune response/Inflammation0.72-0.091.33J05593GRH IRNA for Ig GreceptorFe-receptor0.450.191.37A1623784Thymocyte antigen CD1cAntigen presentation0.400.161.33J05593TIMP-2Immune response/Inflammation0.120.401.17J04641Butyrophilin (BTF3)Unkown function in immune response-0.01-0.051.17J03600LipoxygenaseComplex functions0.020.060.220.13J03692Histone ProteinaseComplex functions0.020.060.220.06J03600LipoxygenaseComplex functions0.020.060.220.06J03600Histone ProteinaseComplex functions incline group0.020.060.220.06J03600Histone ProteinaseComplex functions incline group0.020.060.230.01J03600Histone Proteina	T51594	Neuropilin 1	angiogenesis. regulatory T cells	0.75	0.00	1.73
M989398CD36Ihrombopondin receptor0.440.021.50M28027Thymocyte anigen CD1cAntigen presentation0.270.021.46M32249Unusual 5'-fetal A-gamm-globinCell cycle0.310.410.221.43M29606Interleukin-7 receptor (IL-7)Cytokine receptor0.310.400.131.30M29090g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.72-0.091.33J03909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.72-0.091.33J05593FCRIT mRNA for Ig G receptorFc-receptor0.400.161.33J05593TIMP-2Immune response/ Inflammation0.340.141.27J05593TIMP-2Immune response/ Inflammation0.340.141.27J03054Butyrophilin (BT3)Unknown function in immune response/ Inflammation0.340.141.27J03054Butyrophilin (BT3)No annotation yet0.010.611.01J03600LipoxygenaseCell cycle0.13-0.190.05J0315Gluathione providaseRedox regulation0.050.220.92J04165Histone H1Histone0.060.610.94J05527Histone H1Unknown function in immune response-0.23-0.04-0.34J045621134Otore protein COTTanscription0.050.03-0.34-0.34J05593Histone H1Un	U07802	Tis11d	RNA binding protein	0.49	0.53	1.71
M2827Inymocyte antigen CD1cAntigen presentation0.270.081.45M32249Unusual 5' fetal Agamm-globinCell cycle0.360.140.221.33M29696Interleukin-7 receptor (IL-7)Cytokine receptor0.310.031.40AB01116MahoguninTh2 differentiation0.240.201.39J03909g-interferon-inducible protein (IP-30)Immune response/ Inflamation0.72-0.091.33J05930G-RHI mRNA for Ig GreeptorFc-receptor0.450.191.37Al623784Thymocyte antigen CD1cAntigen presentation0.340.141.27U90548Butyrophilin (BTF3)Unknown function in immune response-0.01-0.051.17M4G2958C21134IAdop958C21134No annotation yet-0.01-0.051.17MAG95958C21134IAdop958C21134Complex functions0.01-0.051.17M4G95958C21134Idtahone prexidaseComplex functions0.060.230.04M1551HCKCell cycle0.13-0.190.96M552Ribonuclease 6 precursorTranscription0.000.050.23M26455GRS proteinIdtahone-0.210.04-0.28M2545GRS proteinCH3Apotosis-0.210.14-0.34M1551HCKApotosis-0.020.04-0.13-0.14M3651HCKApotosis-0.020.04-0.13-0.14M3651	M98398	CD36	Thrombospondin receptor	0.44	0.02	1.50
M32249Unusual 5'-feral A-gamma-globinCell cycle0.360.501.45U71213mGST-1Redox regulation0.410.221.43M29696Interleukin-7 receptor (IL-7)Cytokine receptor0.240.201.39J0390g-interfeno-inducible protein (IP-30)Immune response/Inflammation0.72-0.091.33J0530FcRII mRNA for Ig G receptorC-ceceptor0.450.191.33J0553TIMP-2Antigen presentation0.400.120.391.27J14221Cathepsin B proteinaseAntigen presentation0.410.411.27J05593TIMP-2Unknown function inimune response0.410.411.21J05594Butyrophilin (BTF3)Usknown function inimune response0.01-0.051.18J75593rab 13Complex functions0.01-0.051.18J05602IjobygenaseComplex functions0.01-0.051.01J03600HCKComplex functions0.060.220.90J0315Histone H1Histone0.060.230.91J1315Glutathione peroxidaseRedox regulation0.050.230.91J1437Poto-oncogene protein COTTranscription-0.210.13-0.41J1439Poto-oncogene protein COTTranscription-0.210.13-0.41J1439Poto-oncogene protein COTTranscription-0.310.04-1.12J1439Mithop protei	M28827	Thymocyte antigen CD1c	Antigen presentation	0.27	0.08	1.46
U71213mGST-1Redox regulation0.410.221.43M29696Interlewin-7 receptor (IL-7)Cytokin receptor0.310.030.10M30900g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.72-0.091.33J03909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.72-0.091.33N71535FcKII mRNA for Ig G receptorFc-receptor0.450.161.33J05593TIMP-2Immune response/ Inflammation0.340.141.27M14221Cathepsin B proteinaseMatigen presentation0.340.141.27U90548Butyrophilin (BTF3)Uknown function inmune response/0.010.051.10J03600LipoxygenaseComplex functions0.260.101.05J03600LipoxygenaseComplex functions0.020.001.01J03600Histone ProxidaseRedox regulation0.050.030.01J03700Glutathione peroxidaseRedox regulation0.050.030.01J03115Glotzatione peroxidaseInterlevin function in immune respons0.020.020.02J0433Glutathione peroxidaseInterlevin function in immune respons0.060.020.03J0433Glutathione peroxidaseInterlevin function in immune respons0.060.050.03J0433Glutathione peroxidaseInterlevin function in immune respons0.010.040.14J04	M32249	Unusual 5'- fetal A-gamma-globin	Cell cycle	0.36	0.50	1.45
M29696Interleukin-7 receptor (II-7)Cytokine receptor0.310.031.40AB011116Mahogunin172 differentiation0.720.091.39J03909g-interferon-inducible protein (IP-30)Immune response/Inflammation0.720.091.33N1535FcRII mRNA for Ig G receptorAntigen presentation0.440.161.33Al623784Thymocyte antigen CD1cAntigen presentation0.400.161.33J05593TIMP-2Immune response/Inflammation0.120.991.27M14221Cathepsin B proteinaseAntigen presentation0.340.161.33J05593rab 13Unkown function inmune response-0.010.261.18X75593rab 13Vesicular trafficking0.210.001.17IMAGp958C21134No annotation yet0.01-0.051.10J03600HCKCell cycle0.13-0.190.90X57129Histone H1Histone0.060.220.92X90433Glutathione peroxidaseRedox regulation0.050.230.91U85625Kibonuclease 6 precursorTanscription-0.200.06-0.99N28116GRS proteinUnknown function in immune response-0.210.13-0.94L4457KabpApoptosis-0.200.06-0.93-0.94N28166GRS proteinTanscription-0.210.13-0.94N28161KabponpoptoptinTanscription <td>U71213</td> <td>mGST-1</td> <td>Redox regulation</td> <td>0.41</td> <td>0.22</td> <td>1.43</td>	U71213	mGST-1	Redox regulation	0.41	0.22	1.43
AB011116MahoguninTh2 differentiation0.240.201.39103909g-interfeon-inducible protein (IP-3)Immune response/Inflammation0.72-0.091.39N71535FcRII mRNA for Ig GreeptorAntige presentation0.400.161.33105593TIMP-2Immune response/Inflammation0.120.391.27M1421Cathepsin B proteinaseUnknown function immune response/Inflammation0.120.301.18X75593rab 13Unknown function immune response/Inflammation0.01-0.011.01103604Hayopsilin (BTF3)Unknown function immune response/Inflammation0.01-0.051.10103605rab 13Cell cycle0.01-0.051.01103606Hixone H1Cell cycle0.13-0.050.020.9210353Gluthinio peroxidaseRedox regulation0.050.230.91103606Bibonuclease 6 precursorTranscription0.050.020.9210343Gluthinio peroxidaseApoptosis-0.200.02-0.3110366GelsNarsferi1.03-0.44-0.43-0.4410380Bibrouclease 6 precursorTranscription antistion-0.210.13-0.4410349Poto-oncogene protein COTTranslation-0.210.13-0.4410383Gluthini perspond UMKDNA metabolism0.07-0.22-1.2110439Fotorm C-MAFTranscription antistion-0.48 <t< td=""><td>M29696</td><td>Interleukin-7 receptor (IL-7)</td><td>Cytokine receptor</td><td>0.31</td><td>0.03</td><td>1.40</td></t<>	M29696	Interleukin-7 receptor (IL-7)	Cytokine receptor	0.31	0.03	1.40
J03909g-interferon-inducible protein (IP-30)Immune response/ Inflammation0.72-0.091.33NT1535FcRII mRNA for Ig GreceptorFc-receptor0.650.161.37Al623784Thymocyte antigen CD1cAntigen presentation0.120.391.27M14221Cathepsin B proteinaseAntigen presentation0.340.141.27U90548Butyrophilin (BTF3)Unknown function in immune response/0.010.020.16J03600LipoxygenaseComplex functions in the mune response/0.01-0.051.17J03600LipoxygenaseComplex functions in the mune response/0.01-0.051.01J03600LipoxygenaseComplex functions0.26-0.041.08M16591HGKCell cycle0.13-0.190.96X57129Histone H1Histone0.000.160.90Z3115Icl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune response-0.10.13-0.94J14397Poto-oncogene protein COTTranscription0.000.16-0.93J14318E4BP4Signaling-0.130.04-1.12X64398Nucleolar phosphoproteinTranscription-0.38-0.18-1.22X16396Nucleolar phosphoproteinTranscription-0.38-0.19-1.42X16396Short Gruc C-MAFTranscription-0.38-0.19-1.42X16396	AB011116	Mahogunin	Th2 differentiation	0.24	0.20	1.39
N71535FcRII mRNA for Ig G receptorFc-receptor0.450.191.37A1623784Tymocyte antigen CD1cAntigen presentation0.120.031.13J05593TIMP-2Immune response/ Inflamation0.120.030.121.12M14221Cathepsin B proteinaseAntigen presentation in immune response0.010.021.18X75593rab 13Vesicular trafficking0.210.001.17IMAGp958C21134IMAGp958C21134No annotation yet0.010.051.10J03600LipoxygenaseCell cycle0.130.0190.96X57129Histone H1Hotone0.060.220.921Y0433Glutathione peroxidaseRedox regulation0.050.030.016Z23115bcl-xLApotosic-0.200.01-0.94D1497Proto-oncogene protein COTTranscription-0.210.13-0.94X16396MTHFD2CH3 transfer-0.210.13-0.94X16396MTHFD2DNA metabolism0.07-0.22-1.10X64318EBP4Signaling-0.13-0.94-1.32X4549914Short fort C-MAFTranscription-0.42-0.27-1.27AA496914Short fort C-MAFTranscription-0.38-0.19-1.42X63635Histone C-MAFTranscription-0.38-0.19-1.42X63636Histone C-MAFComplex fortion sincl. cell cycle-0.660.02-1	J03909	g-interferon-inducible protein (IP-30)	Immune response/ Inflammation	0.72	-0.09	1.39
Alfe23784Thymocyte antigen CD1cAntigen presentation0.400.161.33105593TMP-2Immune response/ Inflammation0.120.391.27M1421Cathepsin B proteinaseAntigen presentation0.340.141.271090548Buttyrophilin (BTF3)Unkown function in immune response0.010.020.011.17IMAGp958C21134IMAGp958C21134No annotation yet0.01-0.051.10103000LipoxygenaseComplex functions0.020.010.050.220.92X57129Histone H1Ello complex functions0.060.220.920.92Y00433Glutathione peroxidaseRedox regulation0.050.020.92Y0433bcl-xLApoptosis-0.020.160.90Z5315bcl-xLApoptosis-0.210.13-0.94Y14694Proto-oncogene protein COTTranscription0.07-0.22-1.10Y63355 -terminal region of UMKDNA metabolism0.07-0.22-1.10Y64318EBP4Signaling-0.13-0.14-1.32Y4496914Shortform C-MAFTranscription-0.38-0.19-1.42Y449634Bymphocyte chemoattractant BLCComplex functions incl. cell cycle-0.650.02-1.72Y449634Bymphocyte chemoattractant BLCCell cycle-0.55-0.59-1.75Y46365HSTMCell cycle-0.61-0.51-1.75 <t< td=""><td>N71535</td><td>FcRII mRNA for Ig G receptor</td><td>Fc-receptor</td><td>0.45</td><td>0.19</td><td>1.37</td></t<>	N71535	FcRII mRNA for Ig G receptor	Fc-receptor	0.45	0.19	1.37
J05593TIMP-2Immune response/ Inflammation0.120.391.27M14221Cathepsin B proteinaseAntigen presentation0.340.141.27U90548Butyrophilin (BTS)Unknown function in immune response-0.010.021.18X75593rab 13Vesicular trafficking0.210.001.17J03600LipoxygenaseComplex functions0.02-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Histone0.060.220.92Y00433Glutathione peroxidaseRedox regulation0.000.160.90X23115bcl-xLApoptosis-0.010.06-0.94N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94N1497Proto-oncogene protein COTTranslation-0.210.13-0.94N28416Strommaregin of UMKDNA metabolism-0.77-1.10N28435HABP4Signaling-0.130.04-1.12Z4289Nucleolar phosphoproteinTranscription-0.42-0.27-1.10X64318HABCMGranzme BComplex functions incl. cell cycle-0.38-0.16AF955776Histone C-MAFTranscription incl. cell cycle-0.45-1.45AF765839Michory BComplex functions incl. cell cycle-0.66-1.75X63635Histone C-MAFCamplex functions incl. cell cycle-0.650.02 <td>AI623784</td> <td>Thymocyte antigen CD1c</td> <td>Antigen presentation</td> <td>0.40</td> <td>0.16</td> <td>1.33</td>	AI623784	Thymocyte antigen CD1c	Antigen presentation	0.40	0.16	1.33
M14221Cathepsin B proteinaseAntigen presentation0.340.141.27U90548Butyrophilin (BTF3)Unknown function in immune response-0.010.051.18X75593rab 13Vescular trafficking0.01-0.051.17IMAGp958C21134IMAGp958C21134No annotation yet0.01-0.051.10J03600LipoxygenaseComplex functions0.26-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Redox regulation0.050.230.91U85625Ribonuclease 6 precursorTranscription0.000.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTransfer-0.16-0.31-1.08N28416GRS proteinDNA metabolism-0.07-0.22-1.10X16396MTHFD2CH3 transfer-0.16-0.31-1.08N264318E4BP4Signaling-0.13-0.16-1.12Z34289Nucleolar phosphoproteinTranscription-0.42-0.27-1.27A4496914Short form C-MAFTranscription-0.38-0.18-1.32AF055376Transcription factor C-MAFTranscription-0.21-0.14-1.45A1768839Grazyme BComplex functions incl. cell cycle-0.650.02-1.75X66365JISTIRECell cycle-0.38 <td< td=""><td>J05593</td><td>TIMP-2</td><td>Immune response/ Inflammation</td><td>0.12</td><td>0.39</td><td>1.27</td></td<>	J05593	TIMP-2	Immune response/ Inflammation	0.12	0.39	1.27
U90548Butyrophilin (BTF3)Unknown function in immune response-0.010.261.18X75593rab 13Vesicular trafficking0.210.001.17IMAGp958C21134MAGp958C21134No annotation yet0.01-0.051.10J03600LipoxygenaseComplex functions0.26-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Histone0.060.220.92Y00433Glutathione peroxidaseRedox regulation0.000.160.901Z23115bcl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTranscription-0.21-0.16-0.31-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08D783355 - terminal region of UMKDNA metabolism0.07-0.22-1.10X64318E4BP4Signaling-0.130.04-1.12Z34289Nucleolar phosphoproteinTranscription-0.36-0.18-1.32A406914Short form C-MAFTranscription incl. cell cycle-0.63-0.14-1.32B4557HsMcm6Complex functions incl. cell cycle-0.64-0.14-1.73W80984Blymphocyte chemoattractant BLCChemokine-0.650.02-1.75X66365JLSTIRECell cycle-0.38 </td <td>M14221</td> <td>Cathepsin B proteinase</td> <td>Antigen presentation</td> <td>0.34</td> <td>0.14</td> <td>1.27</td>	M14221	Cathepsin B proteinase	Antigen presentation	0.34	0.14	1.27
X75593rab 13Vesicular trafficking0.210.001.17IMAGp958C21134IMAGp958C21134No annotation yet0.01-0.051.10J03600LipoxygenaseComplex functions0.26-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Histone0.060.220.92Y00433Gluathione perxidaseRedor regulation0.000.160.90U85625Ribonuclease 6 precursorTranscription-0.000.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTranscription-0.16-0.31-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08X2315S-terminal region of UMKDNA metabolism0.07-0.22-1.10X64318H4BP4Signaling-0.38-0.16-1.32X2490Nucleolar phosphoroteinTranscription-0.38-0.14-1.22D44557Transcription Gator C-MAFTranscription-0.38-0.19-1.42D45376HSMCm6Complex functions incl. cell cycle-0.61-0.14-1.73M80984Blymphocyte chemoattractant BLCChemokine-0.630.02-1.75X66365PLSTIRECell cycle-0.38-0.66-1.78W20984JLSTIRECell cycle-0.38-0.66-1.78<	U90548	Butyrophilin (BTF3)	Unknown function in immune response	-0.01	0.26	1.18
IMAGp958C21134IMAGp958C21134No annotation yet0.01-0.051.10J03600LipoxygenaseComplex functions0.26-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Histone0.060.220.92Y00433Glutathione peroxidaseRedox regulation0.050.230.91U85625kibonuclease 6 precursorTranscription0.000.160.90Z23115bcl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune respons-0.23-0.04-0.94D14497Proto-oncogene protein COTTranslation-0.16-0.31-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08D783355 -terminal region of UMKDNA metabolism0.07-0.22-1.10X64318E4BP4Signaling-0.330.04-1.12Z34289Nucleolar phosphoproteinTranscription-0.36-0.18-1.32A496914Short form C-MAFTranscription-0.36-0.19-1.45A1768839Granzyme BCTL-functions incl. cell cycle-0.650.02-1.75W80984B lymphocyte chemoattractant BLCChemokine-0.650.02-1.75W3034JAdp2 homologCell cycle-0.38-0.66-1.78W3035UL D20 LCell cycle-0.38-0.66-1.78W3034 <td>X75593</td> <td>rab 13</td> <td>Vesicular trafficking</td> <td>0.21</td> <td>0.00</td> <td>1.17</td>	X75593	rab 13	Vesicular trafficking	0.21	0.00	1.17
J03600LipoxygenaseComplex functions0.26-0.041.08M16591HCKCell cycle0.13-0.190.96X57129Histone H1Histone0.060.220.92Y00433Glutathione peroxidaseRedox regulation0.050.230.91U85625Ribonuclease 6 precursorTranscription0.000.160.90Z23115bcl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTranslation-0.210.13-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08D783355-terminal region of UMKDNA metabolism0.07-0.22-1.10X64318E4BP4Signaling-0.130.04-1.27AA496914Not form C-MAFTranscription-0.38-0.18-1.32AF055376Transcription factor C-MAFTranscription sincl. cell cycle-0.60-0.19-1.42D84557HsMcm6Complex functions incl. cell cycle-0.650.02-1.75X68398B lymphocyte chemoattractant BLCChemokine-0.650.02-1.75X68365PLSTIRECell cycle-0.38-0.66-1.78X1278Madp2 homologCell cycle-0.27-0.59-1.97	IMAGp958C21134	IMAGp958C21134	No annotation yet	0.01	-0.05	1.10
M16591HCKCell cycle 0.13 -0.19 0.96 X57129Histone H1Histone 0.06 0.22 0.92 Y00433Glutathione peroxidaseRedox regulation 0.05 0.23 0.91 U85625Ribonuclease 6 precursorTranscription 0.00 0.16 0.90 Z23115bcl-xLApoptosis -0.20 0.06 -0.89 N28416GRS proteinUnknown function in immune response -0.23 -0.04 -0.94 D14497Proto-oncogene protein COTTranslation -0.21 0.13 -0.94 X16396MTHFD2CH3 transfer -0.16 -0.31 -1.08 D783355 -terminal region of UMKDNA metabolism 0.07 -0.22 -1.12 X64318E4BP4Signaling -0.13 0.04 -1.12 Z34289Nucleolar phosphoproteinTranscription -0.38 -0.18 -1.32 AF055376Transcription factor C-MAFTranscription -0.38 -0.19 -1.42 D84557HsMcm6Complex functions incl. cell cycle -0.65 0.02 -1.78 W80984B lymphocyte chemoattractant BLCChemokine -0.38 -0.66 -1.78 U31278Madp 2 homologCell cycle -0.72 -0.59 -1.79	J03600	Lipoxygenase	Complex functions	0.26	-0.04	1.08
X57129Histone H1Histone0.060.220.92Y00433Glutathione peroxidaseRedox regulation0.050.230.91U85625Ribonuclease 6 precursorTranscription0.000.160.90Z23115bcl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTranslation-0.210.13-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08D783355 - terminal region of UMKDNA metabolism0.07-0.22-1.10X64318E4BP4Signaling-0.130.04-1.12Z34289Nucleolar phosphoproteinTranscription-0.42-0.27-1.27AA496914Short form C-MAFTranscription-0.38-0.19-1.42D84557HsMcm6Complex functions incl. cell cycle-0.66-0.19-1.42A1768839Granzyme BCTL-function-0.21-0.14-1.73W80984B lymphocyte chemoattractant BLCChemokine-0.650.02-1.75X66365PLSTIRECell cycle-0.38-0.66-1.78U31278Madp homologCell cycle-0.72-0.59-1.97	M16591	НСК	Cell cycle	0.13	-0.19	0.96
Y00433Glutathione peroxidaseRedox regulation0.050.230.91U85625Ribonuclease 6 precursorTranscription0.000.160.90Z23115bcl-xLApoptosis-0.200.06-0.89N28416GRS proteinUnknown function in immune response-0.23-0.04-0.94D14497Proto-oncogene protein COTTranslation-0.210.13-0.94X16396MTHFD2CH3 transfer-0.16-0.31-1.08D783355 -terminal region of UMKDNA metabolism0.07-0.22-1.10X64318E4BP4Signaling-0.130.04-1.12Z34289Nucleolar phosphoproteinTranscription-0.42-0.27-1.27AA496914Short form C-MAFTranscription-0.38-0.19-1.42D84557HsMcm6Complex functions incl. cell cycle-0.60-0.19-1.42A1768839Granzyme BCTL-function-0.21-0.14-1.73W80984Blymphocyte chemoattractant BLCChemokine-0.650.02-1.75X66365LISTIRECell cycle-0.38-0.66-1.78U31278Madp2 homologCell cycle-0.72-0.59-1.97	X57129	Histone H1	Histone	0.06	0.22	0.92
U85625 Ribonuclease 6 precursor Transcription 0.00 0.16 0.90 Z23115 bcl-xL Apoptosis -0.20 0.06 -0.89 N28416 GRS protein Unknown function in immune response -0.23 -0.04 -0.94 D14497 Proto-oncogene protein COT Translation -0.21 0.13 -0.94 X16396 MTHFD2 CH3 transfer -0.16 -0.31 -1.08 D78335 5 -terminal region of UMK DNA metabolism 0.07 -0.22 -1.10 X64318 E4BP4 Signaling -0.13 0.04 -1.12 Z34289 Nucleolar phosphoprotein Transcription -0.36 -0.18 -1.32 AF055376 Transcription factor C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.65 0.02 -1.75 AI768839 Granzyme B CH1-function -0.38 -0.66 -1.78 W30984 B lymphocyte chemoattractant BL	Y00433	Glutathione peroxidase	Redox regulation	0.05	0.23	0.91
Z23115bcl-xLApoptosis -0.20 0.06 -0.89 N28416GRS proteinUnknown function in immune response -0.23 -0.04 -0.94 D14497Proto-oncogene protein COTTranslation -0.21 0.13 -0.94 X16396MTHFD2CH3 transfer -0.16 -0.31 -1.08 D783355 -terminal region of UMKDNA metabolism 0.07 -0.22 -1.10 X64318E4BP4Signaling -0.13 0.04 -1.12 Z34289Nucleolar phosphoproteinTranscription -0.36 -0.18 -1.32 A496914Short form C-MAFTranscription -0.38 -0.19 -1.42 D84557HsMcm6Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839Granzyme BCTL-function -0.21 -0.14 -1.73 W80984B lymphocyte chemoattractant BLCChemokine -0.65 0.02 -1.75 X66365PLSTIRECell cycle -0.38 -0.66 -1.78 U31278Madp2 homologCli cycle -0.72 -0.59 -1.97	U85625	Ribonuclease 6 precursor	Transcription	0.00	0.16	0.90
N28416 GRS protein Unknown function in immune response -0.23 -0.04 -0.94 D14497 Proto-oncogene protein COT Translation -0.21 0.13 -0.94 X16396 MTHFD2 CH3 transfer -0.16 -0.31 -1.08 D78335 5 -terminal region of UMK DNA metabolism 0.07 -0.22 -1.10 X64318 E4BP4 Signaling -0.13 0.04 -1.12 Z34289 Nucleolar phosphoprotein Transcription -0.42 -0.27 -1.27 AA496914 Short form C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.75	Z23115	bcl-xL	Apoptosis	-0.20	0.06	-0.89
D14497 Proto-oncogene protein COT Translation -0.21 0.13 -0.94 X16396 MTHFD2 CH3 transfer -0.16 -0.31 -1.08 D78335 5 -terminal region of UMK DNA metabolism 0.07 -0.22 -1.10 X64318 E4BP4 Signaling -0.13 0.04 -1.12 Z34289 Nucleolar phosphoprotein Transcription -0.36 -0.18 -1.32 A496914 Short form C-MAF Transcription -0.36 -0.18 -1.32 AF055376 Transcription factor C-MAF Transcription incl. cell cycle -0.60 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.197 -1.97	N28416	GRS protein	Unknown function in immune response	-0.23	-0.04	-0.94
X16396 MTHFD2 CH3 transfer -0.16 -0.31 -1.08 D78335 5 -terminal region of UMK DNA metabolism 0.07 -0.22 -1.10 X64318 E4BP4 Signaling -0.13 0.04 -1.12 Z34289 Nucleolar phosphoprotein Transcription -0.42 -0.27 -1.27 AA496914 Short form C-MAF Transcription -0.36 -0.18 -1.32 AF055376 Transcription factor C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	D14497	Proto-oncogene protein COT	Translation	-0.21	0.13	-0.94
D78335 5 -terminal region of UMK DNA metabolism 0.07 -0.22 -1.10 X64318 E4BP4 Signaling -0.13 0.04 -1.12 Z34289 Nucleolar phosphoprotein Transcription -0.42 -0.27 -1.27 A4496914 Short form C-MAF Transcription -0.36 -0.18 -1.32 AF055376 Transcription factor C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	X16396	MTHFD2	CH3 transfer	-0.16	-0.31	-1.08
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AA496914 Short form C-MAF Transcription -0.36 -0.18 -1.32 AF055376 Transcription factor C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	Z34289	Nucleolar phosphoprotein	Transcription	-0.42	-0.27	-1.27
AF055376 Transcription factor C-MAF Transcription -0.38 -0.19 -1.42 D84557 HsMcm6 Complex functions incl. cell cycle -0.60 -0.19 -1.45 AI768839 Granzyme B CTL-function -0.21 -0.14 -1.73 W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	AA496914	Short form C-MAF	Transcription	-0.36	-0.18	-1.32
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W80984 B lymphocyte chemoattractant BLC Chemokine -0.65 0.02 -1.75 X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	AI768839	Granzyme B	CTL-function	-0.21	-0.14	-1.73
X66365 PLSTIRE Cell cycle -0.38 -0.66 -1.78 U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	W80984	B lymphocyte chemoattractant BLC	Chemokine	-0.65	0.02	-1.75
U31278 Madp2 homolog Cell cycle -0.72 -0.59 -1.97	X66365	PLSTIRE	Cell cvcle	-0.38	-0.66	-1.78
	U31278	Madp2 homolog	Cell cycle	-0.72	-0.59	-1.97
D90145 Human LD78 beta gene Chemokine $-0.17 - 0.61 - 1.98$	D90145	Human LD78 beta gene	Chemokine	-0.17	-0.61	-1.98

Table 1. Continued on next page

Table 1. Continued.								
D13639	KIAK0002	Complex functions incl. cell cycle	-0.74	-0.53	-2.13			
J04130	CCL4	Chemokine	-0.85	-0.75	-2.54			
M38193	Granzyme B	CTL-function	0.08	-0.45	-2.92			

Genes for which our quantitative selection criteria (compare Methods) were fulfilled in at least 3 out of 4 combinations of a calcineurin-inhibitor and a TOR-inhibitor are shown. Functional categories are taken from OMIM (Online Mendelian Inheritance in Man) database. A linear statistical model was used to estimate average fold-expression values for entire substance group (CsA and FK for calcineurin-inhibitors, SRL and EVL for TOR-inhibitors, and all four combinations thereof from three donors each) versus control. Results are shown on a log 2 basis. "CI" signifies foldexpression of calcineurin inhibitors, "TI" of TOR-inhibitors and "CI+TI" of the all combinations, respectively.

a consistent but lower difference in gene regulation may have biological significance. However, such small changes in gene expression level are not analyzable with the methods used.

Additive effects in an *in vitro* model may be the result of regulation of transcription or selection of different cell subpopulation in presence of immunosuppressive drugs, especially since the impact of immunosuppressive drugs on T-cell subsets is well documented.⁽²²⁾ Given the short observation period, the latter option seems rather unlikely.

Interestingly, cluster analysis of our data reveals that expression patterns of combined treatment of calcineurin-inhibitors and TOR-inhibitors clearly differ from the patterns of the single drugs. This distinction is caused by supra-additive rather than merely additive effects. The most conspicuous of these "synergisms" describing the transcriptional fingerprint of the drug combination are shown in Table 1.

Among the up-regulated genes many have a function in innate immune response or inflammation. Genes involved in cell cycle, CTL function, chemokines and several transcription factors with a well known role in survival and differentiation of lymphocytes such as E4BP4 (a transcription factor inhibiting apoptosis in pro-B lymphocytes) and c-MAF (a transcription factor involved in TH2-type differentiation) emerged among those genes with synergistic negative gene regulation.

In the above-mentioned study, Cristillo et al. were hoping to find genes regulated specifically by FK or CsA given the differences in structure and binding protein for FK and CsA.⁽²¹⁾ Yet, their quest for substance specific regulation failed possibly because such genes fall under the detection threshold of the c-DNA microarray system. Likewise, we could not detect any substantial differences in regulation between FK and CsA in our system, neither or between SRL and EVL.

Our findings correspond well to published data. For instance, among the single substances only calcineurininhibitors enhance expression of the chaperones CD36 and CD68. In combination of calcineurin-inhibitors with TOR-inhibitors the effect was far more pronounced fulfilling our criteria for synergistic effects on CD36. A similar tendency was seen for CD68 (data not shown). These two scavenger receptors are involved in the uptake of oxidized LDL-cholesterol and possibly participate in the enhanced atherosclerotic risk in transplant recipients. Jin et al.⁽²³⁾ demonstrate an up-regulation of CD36 by CsA which could be verified on protein level for some assay conditions. However, they report different regulation by FK.⁽²⁴⁾ In our system we observed a similar regulation for both genes.

Similarly, in our system monocyte chemoattractant proteins were markedly down regulated by TORinhibitors. Downregulation of monocyte chemoattractant proteins by rapamycin has been extensively studied. The anti-artherogenic effect of rapamycin therapeutically exploited in drug-coated stents is partially attributed to this regulation.⁽²⁵⁾ Diverging effects of combined treatment of calcineurin-inhibitors and TOR-inhibitors versus single drug therapy on scavenger molecules have been reported before.⁽²⁶⁾

To date, there are conflicting data on the effects of the combination of calcineurin-inhibitors and TORinhibitors on lipid metabolism.⁽²¹⁾ It remains to be shown which of the observed effects (down-regulation of monocyte chemoattractant proteins or up-regulation of CD36 and CD68) may prevail *in vivo*.

By cluster analysis we could show that PBL, cultured with immunosuppressants for only 24 hours, clearly vary in their specific fingerprint-like gene expression pattern. In order to define such a "transcriptional fingerprint" we analyzed the "synergistic" effect of the combination of calcineurin-inhibitors and TOR-inhibitors focusing on very strongly supra-additive regulation. We were able to identify a number of genes relevant for either effects or side effects that are specifically regulated by combination of the drugs. It is feasible to assume that such patterns may be used to anticipate the immunosuppressive potential of a certain combination of drugs in a given patient once they have been correlated to clinical data.

Even though the feasibility of deciphering common mechanisms of action of small molecules by gene profiling has been demonstrated,⁽¹⁸⁾ we are aware of the shortcomings of our *in vitro* model. Presumably, some of these effects may be even more pronounced on a post-translational level, at different time points or in purified lymphocyte subsets, while other effects may be overestimated. (INDENT)However, a non hypothesis

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driven approach could lead to a better understanding of pharmacological mechanisms involved and identify potential patterns of biomarkers for effective therapies. The relevance of these patterns will have to be confirmed in correlation to clinical data. If "transcriptional fingerprints" described sufficient immunosuppression or absence of side effects, they could be used to choose the adequate combination of drugs in an individual. Ultimately, knowledge about expression patterns generated by immunosuppressive drugs may help in designing individually "tailored" therapies to render maximum effect and minimize side effects.

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