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Tumor-infiltrating macrophages are associated with metastasis suppression in high-grade osteosarcoma: a rationale for treatment with macrophage-activating agents

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ABSTRACT

PURPOSE: High-grade osteosarcoma is a malignant primary bone tumor with a peak incidence in adolescence. Overall survival of patients with resectable metastatic disease is approximately twenty percent. The exact mechanisms of development of metastases in osteosarcoma remain unclear. Most studies focus on tumor cells, but it is increasingly evident that stroma plays an important role in tumorigenesis and metastasis. We investigated the development of metastasis by studying tumor cells and their stromal context. EXPERIMENTAL DESIGN: To identify gene signatures playing a role in metastasis, we performed genome-wide gene expression profiling on prechemotherapy biopsies of patients who did (n=34) and patients who did not (n=19) develop metastases within five years. Immunohistochemistry was performed on pre-treatment biopsies from two additional cohorts (n=63 and n=16), and on corresponding post-chemotherapy resections and metastases. RESULTS: 118/132 differentially expressed genes were upregulated in patients without metastases. Remarkably, almost half of these upregulated genes had immunological functions, particularly related to macrophages. Macrophage associated genes were expressed by infiltrating cells and not by osteosarcoma cells. Tumor-associated macrophages (TAMs) were quantified with immunohistochemistry and were associated with significantly better overall survival in the additional patient cohorts. Osteosarcoma samples contained both M1 (CD14/HLA-DRα positive) and M2 type TAMs (CD14/CD163 positive and association with angiogenesis). CONCLUSION: In contrast to most other tumor types, TAMs are associated with reduced metastasis and improved survival in high-grade osteosarcoma. This study provides a biological rationale for the adjuvant treatment of high-grade osteosarcoma patients with macrophage-activating agents, such as muramyl tripeptide.

INTRODUCTION

High-grade osteosarcoma is a malignant bone tumor characterized by the production of osteoid. The highest incidence is in adolescent patients, with a second peak in patients over 40 years of age [214]. Despite wide-margin surgery and intensification of chemotherapeutic treatment, overall survival rates have reached a plateau at about sixty percent [13;24;141]. Novel treatment modalities are needed, but data on critical biological mechanisms allowing the development of novel therapeutic agents are scarce for this relatively rare tumor. In addition to conventional chemotherapeutic agents, recent trials have explored immunostimulatory strategies. The ongoing EURAMOS-1 trial randomizes for treatment with interferon (IFN)-α in patients with good histological response to neo-adjuvant chemotherapy [160]. A recently published clinical trial has shown improved overall survival for osteosarcoma patients treated with the macrophage activating agent muramyl tripeptide (MTP) added to the standard chemotherapy regimen [170]. However, only limited information on macrophage infiltration and activation in osteosarcoma is available [126].

Tumor-associated macrophages (TAMs) may promote tumorigenesis through immunosuppression, expression of matrix-degrading proteins and support of angiogenesis. In numerous cancer types, high numbers of M2 or 'alternatively activated' TAMs are associated with a worse prognosis [93;112;138;146;266;273]. M2 macrophages have important functions in wound-healing and angiogenesis, express high levels of the immunosuppressive cytokines IL-10 and TGF-β and express scavenger receptors such as CD163 [210;237]. 'Classical activation' of macrophages by interferon-γ or microbial products results in polarization towards M1 type macrophages. M1 macrophages express high levels of pro-inflammatory cytokines such as interleukin (IL)-12 , IL-1, and IL-6 and have potent anti-tumor efficacy, both by reactive oxygen species and cytokine-induced cytotoxicity and by induction of natural killer (NK) and T cell activity [188]. Rarely, high numbers of TAMs are associated with better prognosis [73;122]. In these cases, TAMs are presumably polarized towards an M1 phenotype, although macrophage subtypes were not reported in these two studies. Alternatively, macrophages may directly phagocytose tumor cells, as has been demonstrated in acute myeloid leukemia [109].

To investigate the role of stroma and stroma-tumor interactions important in metastasis of osteosarcoma, we investigated the development of metastasis by studying tumor cells and their stromal context. Using genome-wide expression analysis, we showed that high expression of macrophage-associated genes in pre-treatment biopsies was associated with a lower risk of developing metastases. In addition, we quantified and characterized TAMs in two independent cohorts, including pre-treatment biopsies, post-chemotherapy resections, and metastatic lesions. In contrast to the tumor-supporting role for TAMs in most epithelial tumor types, higher numbers of infiltrating TAMs correlated with better survival in osteosarcoma. Our findings suggest that macrophages have direct or indirect anti-osteosarcoma activity and provide a possible explanation for the beneficial effect of treatment with macrophage activating agents in osteosarcoma.

MATERIALS AND METHODS

Patient cohorts

Genome-wide expression profiling was performed on snap-frozen pre-treatment diagnostic biopsies containing viable tumor material of 53 resectable high-grade osteosarcoma patients from the EuroBoNet consortium (http://www.eurobonet.eu) (cohort 1). For immunohistochemical validation a tissue microarray containing 145 formalin-fixed paraffin-embedded (FFPE) samples of 88 consecutive high-grade osteosarcoma patients with primary resectable disease (cohort 2) and 29 FFPE samples of a cohort of 20 consecutive high-grade osteosarcoma patients with resectable disease were used (cohort 3), including material from pre-treatment biopsies, postchemotherapy resections, and metastatic lesions [183]. Clinicopathological details can be found in Suppl. Table 4.1. All biological material was handled in a coded fashion. Ethical guidelines of the individual European partners were followed and samples and clinical data were stored in the EuroBoNet biobank.

Cell lines

The nineteen osteosarcoma cell lines HAL, HOS, HOS-143b, IOR/MOS,IOR/OS10, IOR/OS14, IOR/OS15, IOR/OS18, IOR/OS9, KPD, MG-63, MHM,MNNG-HOS, OHS, OSA, Saos-2, SARG, U2OS, and ZK-58 were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum and 1% Penicillin/Streptomycin (Invitrogen) as previously described [199].

RNA isolation, cDNA synthesis, cRNA amplification, and Illumina Human-6 v2.0 Expression BeadChip hybridization

Osteosarcoma tissue was snap-frozen in 2-Methylbutane (Sigma-Aldrich, Zwijndrecht, the Netherlands) and stored at -70°C. Using a cryostat, 20 µm sections from each block were cut and stained with hematoxylin and eosin to ensure at least 70% tumor content and viability. RNA was isolated using TRIzol (Invitrogen), followed by RNA clean-up using the QIAGEN Rneasy mini kit with on-column DNAse treatment (Venlo, the Netherlands). RNA quality and concentration were measured using an Agilent 2100 Bioanalyzer (Santa Clara,CA, USA) and Nanodrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA), respectively. Synthesis of cDNA, cRNA amplification, and hybridization of cRNA onto the Illumina Human-6 v2.0 Expression BeadChips (San Diego, CA, USA) were performed as per manufacturer's instructions. Reverse transcriptase quantative polymerase chain reaction (RT-qPCR) RT-qPCR analysis of selected target genes was performed as previously described [220]. Each experiment was performed in duplicate using an Automated Liquid-Handling System (Tecan, Genesis RSP 100, Männedorf, Switzerland). Data were normalized using geometric mean expression levels of three reference genes, *i.e. SRPR*, *CAPNS1*, and *TBP* using geNorm (http://medgen.ugent.be/~jvdesomp/genorm/). Primer sequences can be found in Suppl. Table 4.2.

Enzymatic and fluorescent immunostainings

Enzymatic and fluorescent immunostainings were performed on 4 µm sections of FFPE tissue as previously described [183]. Details regarding antibodies and procedures can be found in

Suppl. Table 4.3. In case of double immunohistochemistry (IHC), incubation with anti-CD45 and development with DAB+ (Dako, Glostrup, Denmark) occurred first, followed by a second antigen retrieval before incubation with either anti-CD163 or anti-HLA-DRα and development using the alkaline-phosphatase substrate Vector Blue (Vector Labs, Burlingam, CA, USA). In case of double immunofluorescent (IF) stainings, primary antibodies were co-incubated overnight. As a positive control normal and formic acid decalcified tonsil was used, as a negative control no primary antibody was added. Tissue microarray slides were scanned using the MIRAX SCAN slide scanner and software (Zeiss, Mirax 3D Histech, Hungary). Numbers of positively stained cells and vessels were counted using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) and averaged per 0.6 mm core. IF and double IHC images were acquired using a Leica DM4000B microscope (Wetzlar, Germany) fitted with a CRI Nuance spectral analyzer (Cambridge Research and Instrumentation Inc., Woburn, MA, USA) and analyzed using the supplied co-localization tool to determine percentage of single and double positive pixels per region of interest.

Microarray data analysis

Gene expression data were exported from BeadStudio version 3.1.3.0 (Illumina) in GeneSpring probe profile format and processed and analyzed using the statistical language R [211]. As Illumina identifiers are not stable and consistent between different chip versions, raw oligonucleotide sequences were converted to nuIDs [59]. Data were transformed using the variance stabilizing transformation algorithm to take advantage of the large number of technical replicates available on the Illumina BeadChips [145]. Transformed data were normalized using robust spline normalization, an algorithm combining features of quantile and loess normalization, specifically designed to normalize variance stabilized data. All microarray data processing was carried out using Bioconductor package lumi [60;84]. Quality control was performed using Bioconductor package arrayQualityMetrics [119]. MIAME-compliant data have been deposited in the GEO database (www.ncbi.nlm.nih.gov/geo/, accession number GSE21257).

Statistical analysis

Differential expression between patients who did (n=34) and did not (n=19) develop metastases within five years from diagnosis of the primary tumor was determined using Linear Models for Microarray Data (LIMMA),[240] applying a Benjamini and Hochberg False Discovery Rate adjusted *P*-value cut-off of 0.05. Other univariate statistical analyses were performed using GraphPad Prism software (version 5.01, La Jolla, California, USA). Multivariate survival analyses were carried out according to the Cox proportional hazards model in SPSS (version 16.0.2, Chicago, Illinois, USA). Two-sided *P*-values <0.05 were determined to be significant; *P*-values between 0.05 and 0.15 were defined to be a trend.

RESULTS

High expression of macrophage-associated genes in osteosarcoma biopsies of patients who did not develop metastases within five years from diagnosis (cohort 1)

Comparison of genome-wide gene expression in tumors of patients who did and did not develop metastases within five years resulted in 139 significantly differentially expressed (DE) probes, of which 125 corresponded to 118 upregulated and 14 to downregulated genes in patients who did not develop metastases. A summary of DE genes and detailed descriptions of all probes can be found in Table 4.1 and Suppl. Table 4.4, respectively. Two DE genes were specific for macrophages (*CD14* and *MSR1*) and 30/132 of the DE genes were associated with macrophage functions such as antigen processing and presentation (*e.g. HLA-DRA* and *CD74*) or pattern recognition (*e.g. TLR4* and *NLRP3*). Overall, approximately 20% of the upregulated probes corresponded to genes which were associated with macrophage function and development and an additional 25% of the upregulated probes corresponded to genes with other immunological functions, such as cytokine production and phagocytosis. Four genes were selected for validation of the microarray data using RT-qPCR: *CD14*, *HLA-DRA*, *CLEC5A*, and *FCGR2A*. Expression levels as determined by RTqPCR correlated well with expression levels obtained by microarray analysis (Suppl. Fig. 4.1). Metastases-free survival curves of the same cohort, generated using median expression of the probe of interest as a cut-off determining low and high expression, are shown in Fig. 4.1b and in Suppl. Fig. 4.2. Cox proportional hazards analysis revealed expression of macrophage-associated genes *CD14* and *HLA-DRA* to be independently associated with metastasis free survival (Suppl. Table 4.5).

Macrophage-associated genes are expressed by infiltrating hematopoietic cells and not by tumor cells

The most probable source of expression of the differentially expressed macrophage-associated genes were infiltrating immune cells and not osteosarcoma cells. To confirm this, we performed qRT-PCR of *CD14* and *HLA-DRA* on osteosarcoma cell lines (n=19) and biopsies (n=45, a subset of cohort 1). *CD14* and *HLA-DRA* expression was variable in osteosarcoma biopsies, but almost undetectable in cell lines. This indicates that these macrophage-associated genes were not expressed by tumor cells but by infiltrating cells, since only osteosarcoma biopsies contain macrophage infiltrate, whereas RNA from cell lines is exclusively from tumor cells (Fig. 4.1a, *P*-value Mann-Whitney U test <0.0001). In addition, we performed double IHC for the hematopoietic cell marker CD45, which is not expressed by osteosarcoma tumor cells, and the macrophage marker CD163 or the macrophage-associated protein HLA-DRα (Fig. 4.1c). We chose this approach because no reliable osteosarcoma markers are available (1). Our results confirmed that infiltrating, hematopoietic cells were the source of the macrophage-associated gene expression levels. Together, these data show that osteosarcoma tumor cells do not express macrophage-associated genes, neither *in vitro* nor *in vivo*.

Table 4.1 Differentially expressed genes and probes by category comparing high-grade osteosarcoma patients with and without metastases within five years by genome-wide expression profiling (cohort 1). Twenty percent of differentially expressed probes corresponded to genes which are associated with macrophage functions such as antigen processing and presentation or pattern recognition. Twenty-five percent of the upregulated probes corresponded to genes with other immunological functions, such as cytokine production and phagocytosis.

	Higher expression in patients without metastases			Lower expression in patients without metastases		
Category	**Nr of probes**	**Nr of genes**	**Examples**	**Nr of probes**	**Nr of genes**	**Examples**
Pattern recognition receptor or signaling	18	17	*MSR1, CD14, NLRP3, TLR7, TLR8, TLR4, NAIP, IL1B, PYCARD, NLRC4*	0	0	
Immunological	16	15	*CD86, C1QA, LY9, CD37, LY86*	0	0	
HLA class II	12	12	*HLA-DMB, HLA-DRA, CD74, HLADQA1*	0	0	
Hematopoietic cells	11	10	*HMHA1, MYO1G, LST1*	0	0	
Cytokines and cytokine signaling	7	6	*CXCL16, CSF2RA, IFNGR1, IL10RA*	1	1	*MAP2K7*
Metabolism	9	9	*PFKFB2, SLC2A9, CECR1, ALOX5*	0	0	
Fc receptor	6	4	*FCGR2B, FCGR2A, FGL2, PTPN6*	0	0	
Cytoskeleton	5	5	*HCLS1, WAS, IQGAP2*	1	1	*DNAI2*
(An)ion transporters and channels	4	4	*SLCO2B1, SLC11A1*	1	1	*SLC24A4*
AKT pathway	3	3	*PIK3IP1, PKIB*	0	0	
Endocytosis	3	3	*APPL2, NECAP2*	0	0	
Apoptosis, cell cycle control and proliferation	4	4	*TMBIM4, TNFRSF1B, OGFRL1*	1	1	*BCCIP*
Signaling	4	4	*RGS10, MFNG, FHL2, PILRA*	0	0	
Growth hormone signaling	0	0		1	1	*GHR*
Morphogenesis	0	0		1	1	*HOXC4*
Others	7	6	*CUGBP2, CYP2S1, VAV1, GGN*	2	2	*NSUN5, MRPL4*
Unknown	16	16	*VMO1, MICALCL, MS4A6A*	6	6	*NHN1, BRWD1*
Total	125	118		14	14	

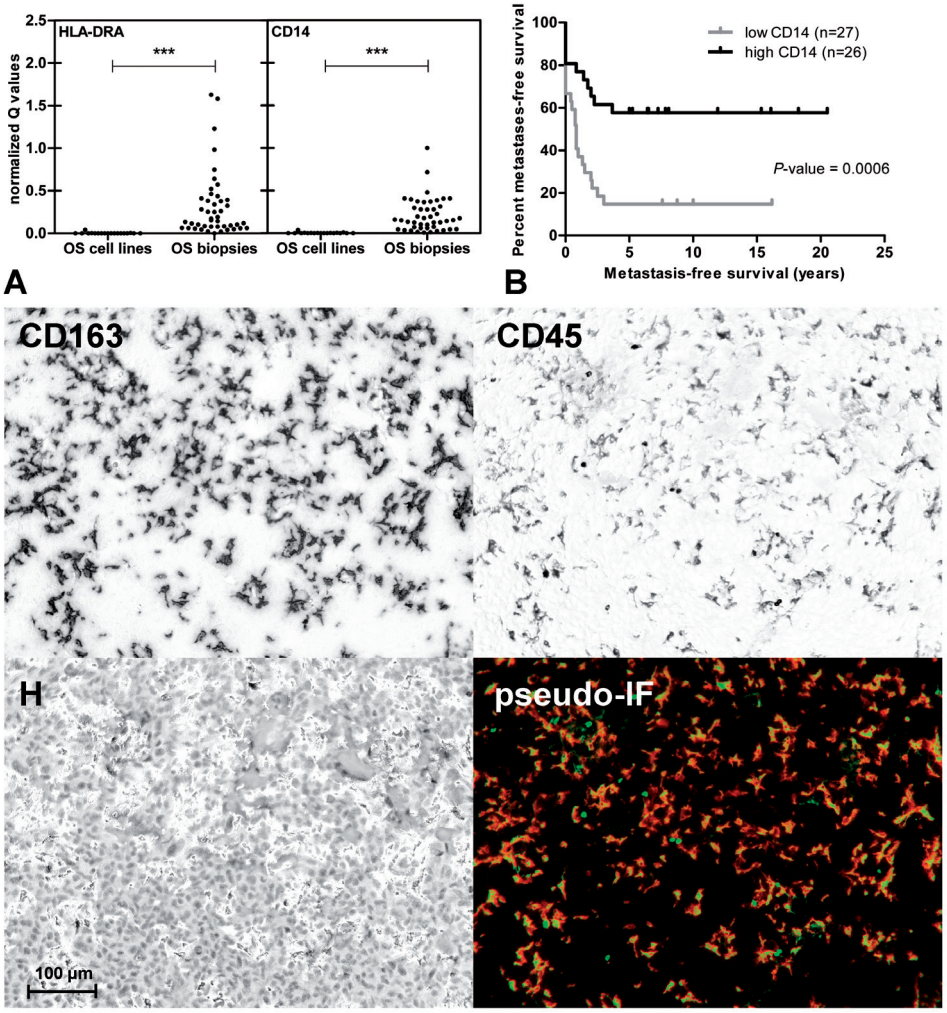

Fig. 4.1 Macrophage-associated genes are not expressed by osteosarcoma tumor cells. **a**, RT-qPCR of osteosarcoma cell lines and biopsies of *CD14* and *HLA-DRA* demonstrating lack of expression by osteosarcoma cells. *P*-value Mann-Whitney U test <0.0001 noted as ***. **b**, high expression of macrophage associated genes was associated with a better metastasis-free survival (cohort 1, Kaplan-Meier curve, *P*-value obtained using logrank test, patients with metastasis at diagnosis have an event at t=0. These patients are included, because patients who develop metastases later on may as well have micrometastases at time of diagnosis. Metastasis-free survival curves for *HLA-DRA*, *CLEC5A*, and *FCGR2A* can be found in Supplemental Figure 2. **c**, double immunohistochemical staining of CD163 with the hematopoietic cell marker CD45 was performed with haematoxylin counterstain (H) and analyzed using spectral imaging microscopy. The pseudo-immunofluorescent image (pseudo-IF) shows CD163 positive cells in red, CD45 positive cells in green and co-localization of both markers in orange. Lack of expression of CD163 and CD45 on surrounding tumor cells (dark blue) and some single positive CD45 cells can be noted.

Macrophage numbers in osteosarcoma biopsies correlate with *CD14* gene expression levels and are positively associated with localized disease and better outcome (cohorts 2 and 3)

To confirm the presence of TAMs in osteosarcoma we stained a tissue microarray containing 145 samples of 88 patients for the macrophage marker CD14 and counted the number of positive cells per tissue microarray core (cohort 2, Fig. 4.2a). CD14 was chosen as opposed to CD68 because the latter marker is not expressed by monocytes and often shows cross-reactivity with mesenchymal tissue (data not shown). Number of CD14 positive cells per tissue microarray core correlated significantly with *CD14* mRNA expression levels (14 samples overlap with gene expression analysis, Spearman correlation coefficient 0.64, *P*-value = 0.01). Similar to the gene expression data, there was a trend for patients with primary localized disease to have higher numbers of macrophages in pre-treatment diagnostic biopsies than patients with metastatic disease at presentation (mean number of macrophages per core 55 *vs*. 27, Mann-Whitney U test *P*-value 0.09). Also, patients with high macrophage counts at diagnosis tended to be less likely to develop metastases within five years (χ^2 *P*-value 0.13). We subdivided this cohort into four quartiles based on numbers of CD14 positive cells in order to determine the group with the best overall survival. No significant differences were found between quartiles 2-4, but patients beloning to this group had better overall survival as compared to patients with low CD14 counts (lowest quartile, or less than 12 CD14 positive cells per tissue array core, Fig. 4.2b, *P*-value log-rank test = 0.02). In another cohort of 16 patients, IF staining of CD14, CD163 and HLA-DRα was performed, again confirming a potential prognostic value of high macrophage numbers (cohort 3, Fig. 4.3, *P*-value log-rank test = 0.01 and Suppl. Fig. 4.3).

Macrophages in osteosarcoma have both M1 and M2 characteristics

To determine the phenotype of macrophages present in osteosarcoma, we performed double IHC with CD14 and either the M1-associated marker HLA-DRα or the M2-associated marker CD163. Not all CD163 and HLA-DR positive infiltrating cells expressed CD14 (Fig. 4.3a and Suppl. Fig. 4.3a). The total number of macrophages as determined by quantifying CD14 positive macrophages was associated with good survival (Fig. 4.3b), but the phenotype of the macrophages (CD14/CD163 double positive versus CD14/HLA-DRα double positive) was not (Suppl. Fig. 4.3b and data not shown). Another M2 characteristic is support of angiogenesis. The number of CD14 positive macrophages correlated with the number of CD31 positive vessels (Fig. 4.2a and Fig. 4.4), but vascularity did not correlate with prognosis (data not shown).

Macrophage numbers in diagnostic biopsies may predict histological response to chemotherapy and macrophage number increases following chemotherapy treatment

There was a trend for high macrophage count (highest three quartiles or >12 CD14-positive cells per tissue array core) in pre-chemotherapy diagnostic biopsies of the primary tumor to predict for good histological response to neoadjuvant chemotherapy (defined as more than 90% non-vital tumor tissue upon final resection), since 46% of patients with high macrophage

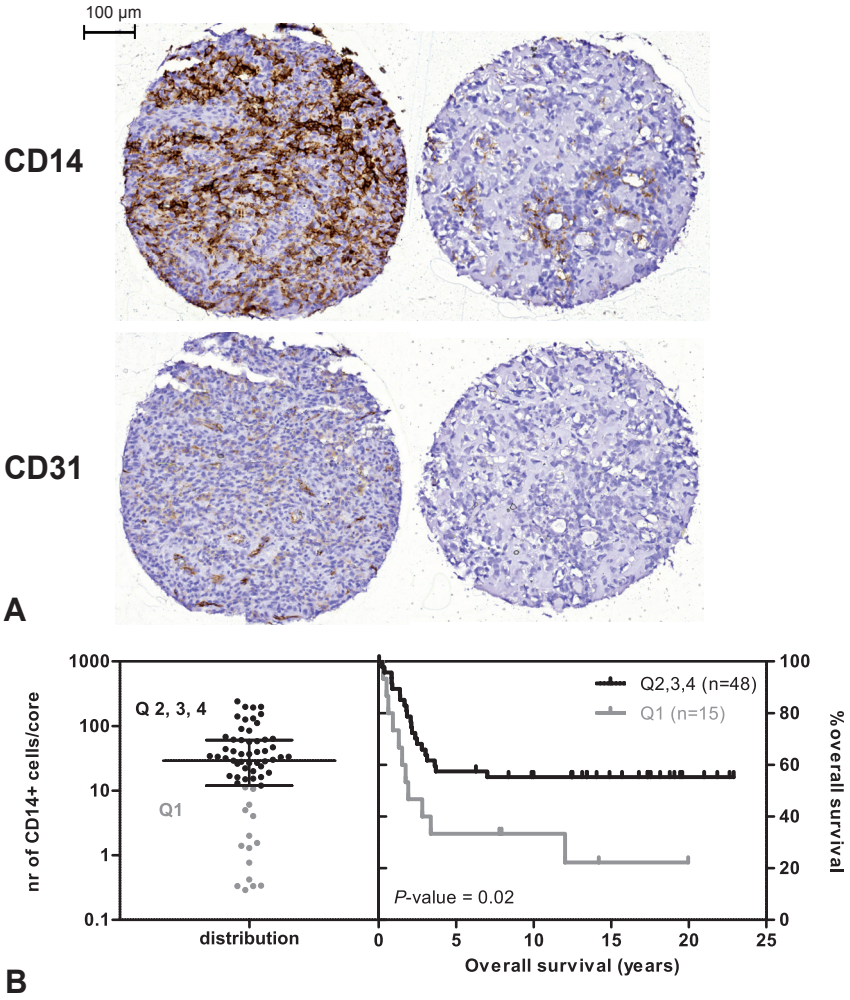

Fig. 4.2 **a**, example of representative stainings of high-grade osteosarcoma with high (left panels) versus low (right panels) levels of macrophage infiltration (CD14 staining) and vascular density (CD31 staining). **b**, high numbers of infiltrating macrophages (left panel, defined as the three upper quartiles, or more than twelve CD14 positive cells per tissue array core) are associated with better overall survival (right panel, *P*-value logrank test = 0.02, cohort 2). Q1, lowest quartile; Q2, 3, 4, three highest quartiles.

numbers and 18% of patients with low macrophage numbers had a good histological response (cohort 2; χ^2 *P*-value 0.09). The prognostic benefit of macrophage counts in osteosarcoma was not independent of histological response using Cox proportional hazard analysis. Macrophage numbers were higher in post-chemotherapy resections of the primary tumor as compared to the pre-chemotherapy biopsies (Suppl. Fig. 4.4). Moreover, gene expression analysis showed upregulation of macrophage-associated probes in post-chemotherapy resections (n=4) as compared with pre-chemotherapy biopsies (n=79, data not shown).

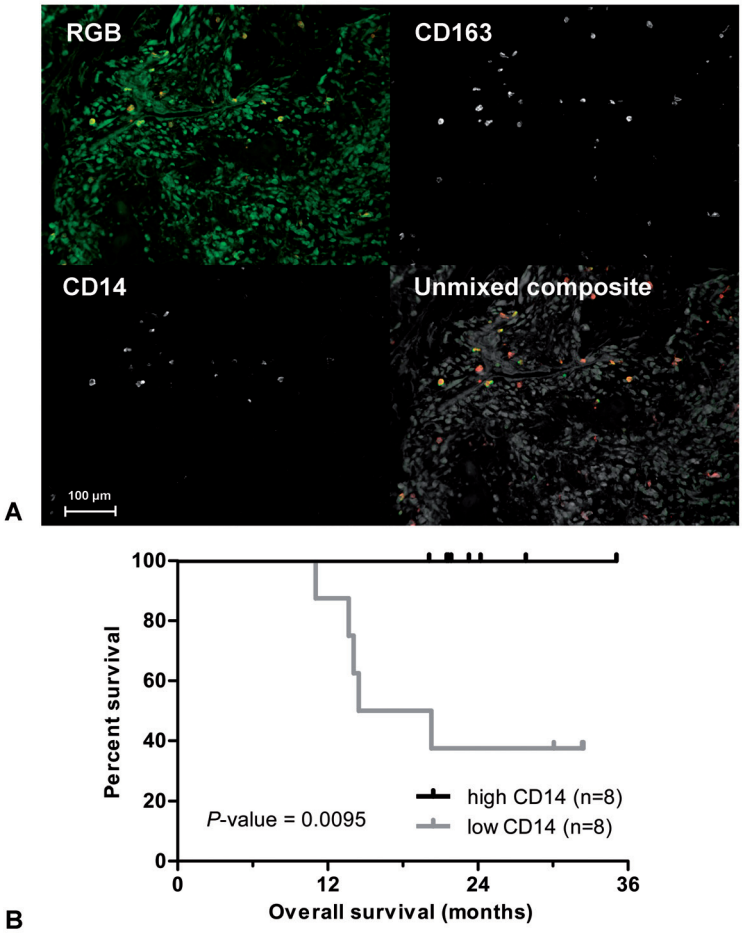

Fig. 4.3 **a**, osteosarcoma samples are infiltrated with CD14 and CD163 single and double positive macrophages. Spectral imaging was used to reduce auto-fluorescence of osteosarcoma cells. In the composite image, CD14 positive cells are represented in green, CD163 positive cells are represented in red, and CD14/CD163 double positive cells are represented in yellow. Background auto-fluorescence of tumor cells is represented in grey. **b**, in an independent cohort of 16 patients (cohort 3), high macrophage infiltration as determined by immunofluorescent CD14 staining was associated with significantly improved overall survival. *P*-values obtained using logrank test, cut-off at the median.

DISCUSSION

Overall survival of high-grade osteosarcoma patients with resectable metastatic disease is poor at about twenty percent [32]. Mechanisms for the development of metastases in osteosarcoma are elusive. To identify genes that play a role in this process, we performed genome-wide expression profiling on pre-chemotherapy biopsies of osteosarcoma patients. We compared patients who developed clinically detectable metastases within five years with patients who did not develop metastases within this time frame (cohort 1). About 20% percent of genes overexpressed in

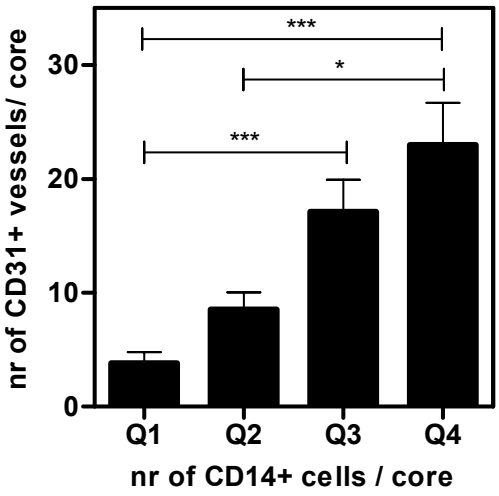

Fig. 4.4 Macrophage infiltration as determined by CD14 positive cell count correlated with vascularity as determined by CD31 positive vessel count. Data of all osteosarcoma samples (pre- and post-treatment primary tumor and metastatic samples, cohort 2) is shown. Q1, lowest quartile; Q2, 3, 4, three highest quartiles. Kruskal-Wallis test $P < 0.0001$, Dunn's post-test P-values <0.05 noted as *, <0.001 noted as ***.

patients without metastases were macrophage-associated, while an additional 25% percent of genes had other immunological functions (for example in phagocytosis, complement activation or cytokine production and response) but could still be attributed to macrophages (Table 4.1 and Suppl. Table 4.4). Thus, in total almost half of the differentially expressed genes belonged to one specific process, *i.e.* macrophage function. Macrophage-associated genes were expressed by infiltrating hematopoietic cells and not by osteosarcoma tumor cells (Fig. 4.1), indicating a possible role for macrophages in preventing metastasis in osteosarcoma. To confirm these findings, we quantified infiltrating macrophages in two additional cohorts (cohort 2 and 3) and found an association with better overall survival in both cohorts.

The anti-metastatic effect of TAMs in osteosarcoma is remarkable, since TAMs support tumor growth in a substantial number of other cancers, which are mostly tumors of epithelial origin. For example, macrophages are associated with the angiogenic switch in breast cancer [144]. We find an association between macrophage infiltration and higher microvessel density, which suggests that the influx of macrophages may support certain aspects of tumor growth in osteosarcoma as well. However, in the case of osteosarcoma, direct or indirect anti-tumor activity of macrophages apparently outweighs their possible tumor-supporting effects. Macrophages can alter their phenotype from M2 to M1 and become the tumor's foe instead of its friend, given the right circumstances [35;92;239]. The TAMs that were identified in this study in osteosarcoma had both M1 and M2 characteristics. The expression of CD163 and the association with angiogenesis are M2 characteristics [144;195]. Some of the differentially expressed genes, such as *MSR1* and *MS4A6A* are specific for M2 macrophages *in vitro* [161]. Others, such as the pro-inflammatory cytokine *IL1B* are more indicative of an M1 phenotype

[188]. How macrophages inhibit osteosarcoma metastasis and if a balance between M1 and M2 type functions is responsible, is unknown.

In a multivariate regression model, the survival benefit of high TAM numbers was at least partly dependent on histological response to chemotherapy. Chemotherapy can cause "immunogenic cell death" of cancer cells, resulting in the release of endogenous danger signals [129;288]. The binding of these danger signals to pattern recognition receptors on macrophages can skew polarization of M2 to M1 type TAMs. The interaction between dying tumor cells and resident TAMs may facilitate clearance or inhibit outgrowth of metastatic tumor cells. However, patients with localized disease at diagnosis tended to have a larger macrophage infiltrate than patients with metastatic disease at diagnosis (mean number of macrophages per core 55 *vs*. 27). At this point, patients have not undergone chemotherapeutic treatment yet and an interaction between chemotherapy and macrophages can therefore not be responsible for the anti-metastatic effect of macrophages. Perhaps the anti-metastatic effect of TAMs in these patients is due to the constitutive presence of macrophages with an M1 phenotype. Alternatively, the presence of macrophages might be a reflection of a microenvironment not conducive for metastasis. Although preliminary analysis of a clinical trial investigating the effect of treatment with the macrophage activating agent MTP yielded conflicting results, subsequent analysis revealed that treatment with MTP improved six-year overall survival from 70 to 78% in a cohort of patients with primary localized disease [169;170]. Similar results were obtained in canine osteosarcoma [134]. MTP is a synthetic derivative of muramyl dipeptide (MDP), a common bacterial cell wall component. Muropeptides bind to intracellular pattern recognition receptors of the nucleotide-binding and oligomerization domain (NOD) like (NLR) family, expressed by macrophages [82]. In our study, five genes associated with NLR family signaling and the associated 'inflammasome' were highly expressed in pre-treatment biopsies of patients who do not develop metastases. The differentially expressed genes *NLRP3*, *NAIP*, *NLRC4* and *PYCARD* are components of the inflammasome, *LYZ* is a lysozyme which processes bacterial cell wall peptidoglycan into muramyl dipeptide, a ubiquitous natural analogue of MTP and *IL1B* is the downstream effector cytokine of the inflammasome pathway. Further research is needed to clarify if only patients with high numbers of TAMs benefit from MTP treatment, or if MTP treatment is effective regardless of macrophage number or activation status pre-treatment. Also, it is unknown if treatment with agents promoting macrophage migration or with other macrophage activating agents like toll-like receptor ligands or interferons have a similar beneficial effect on outcome.

Previous genome-wide expression profiling studies in osteosarcoma focused on identifying genes that predict histological response to neo-adjuvant chemotherapy [153;176;194;229]. As a consequence, the importance of macrophages in controlling metastases was not recognized. However, we previously compared gene expression profiles of osteosarcoma biopsies and cultured mesenchymal stem cells and determined which genes are expressed by tumor stroma and not by tumor cells [47]. There is considerable overlap between the stromal genes identified in our previous study and the macrophage-associated genes identified in the present study (including HLA class II genes as the most prevalent differentially expressed group of genes and the macrophage-associated genes *MSR1*, *MS4A6A*, and *FCFGR2A*).

In conclusion, we demonstrated the presence and clinical significance of TAMs in pre-treatment samples of high-grade osteosarcoma. TAMs in osteosarcoma are a heterogeneous cell population with both M1 anti-tumor and M2 pro-tumor characteristics. Although the exact mechanism by which macrophages exert their anti-metastatic functions is still unknown, this study provides an important biological rationale for the treatment of osteosarcoma patients with macrophage activating agents.

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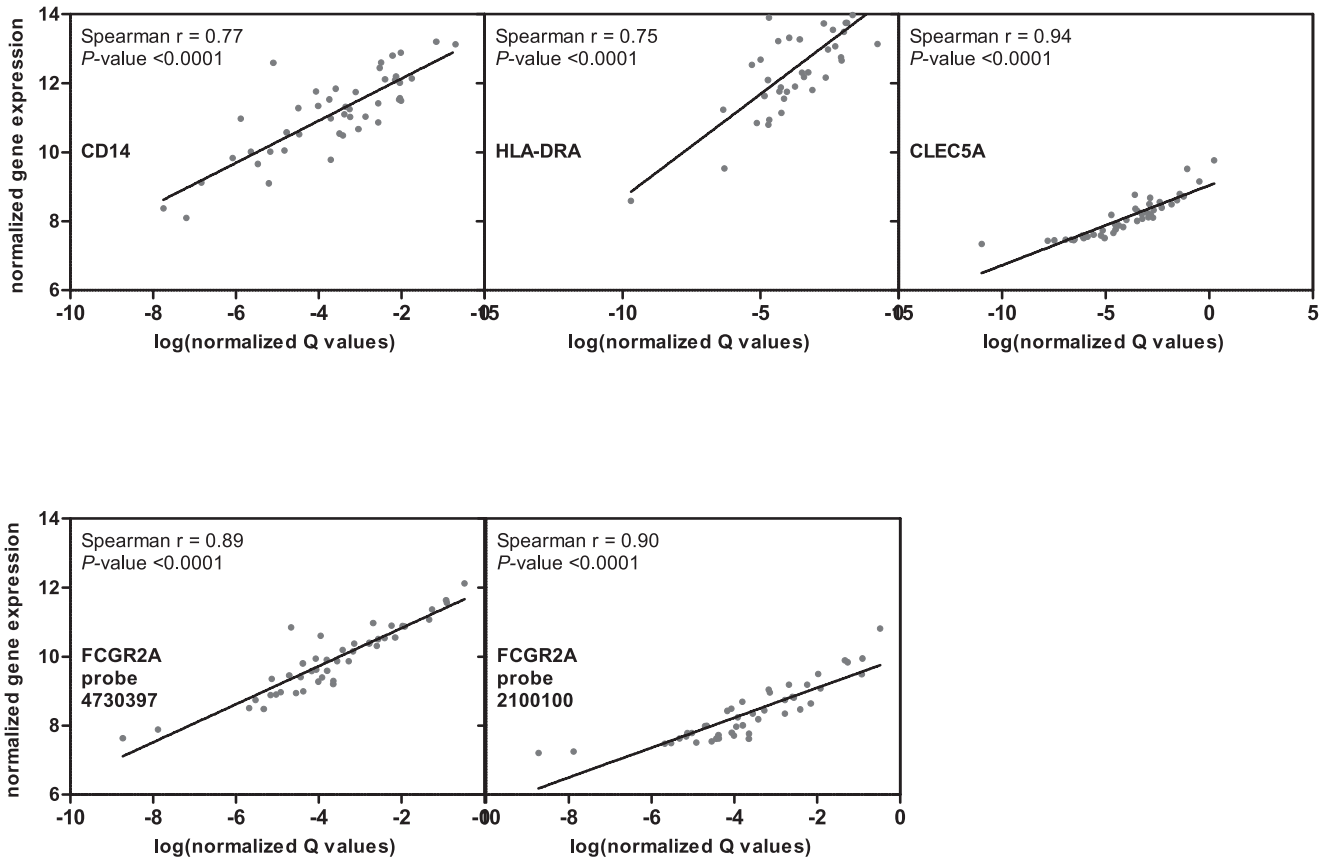
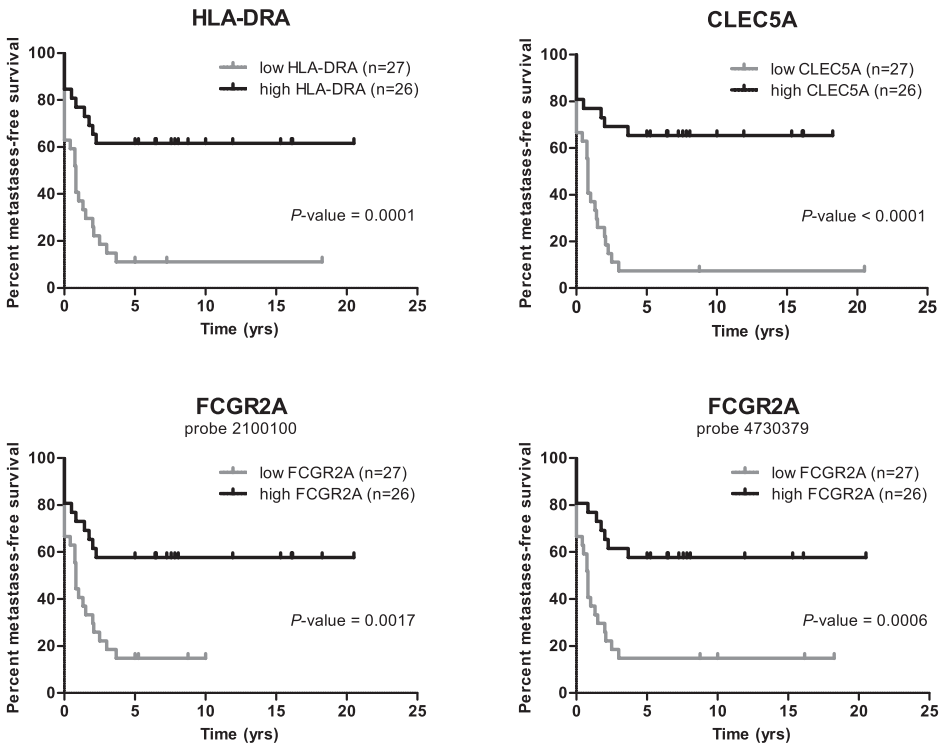
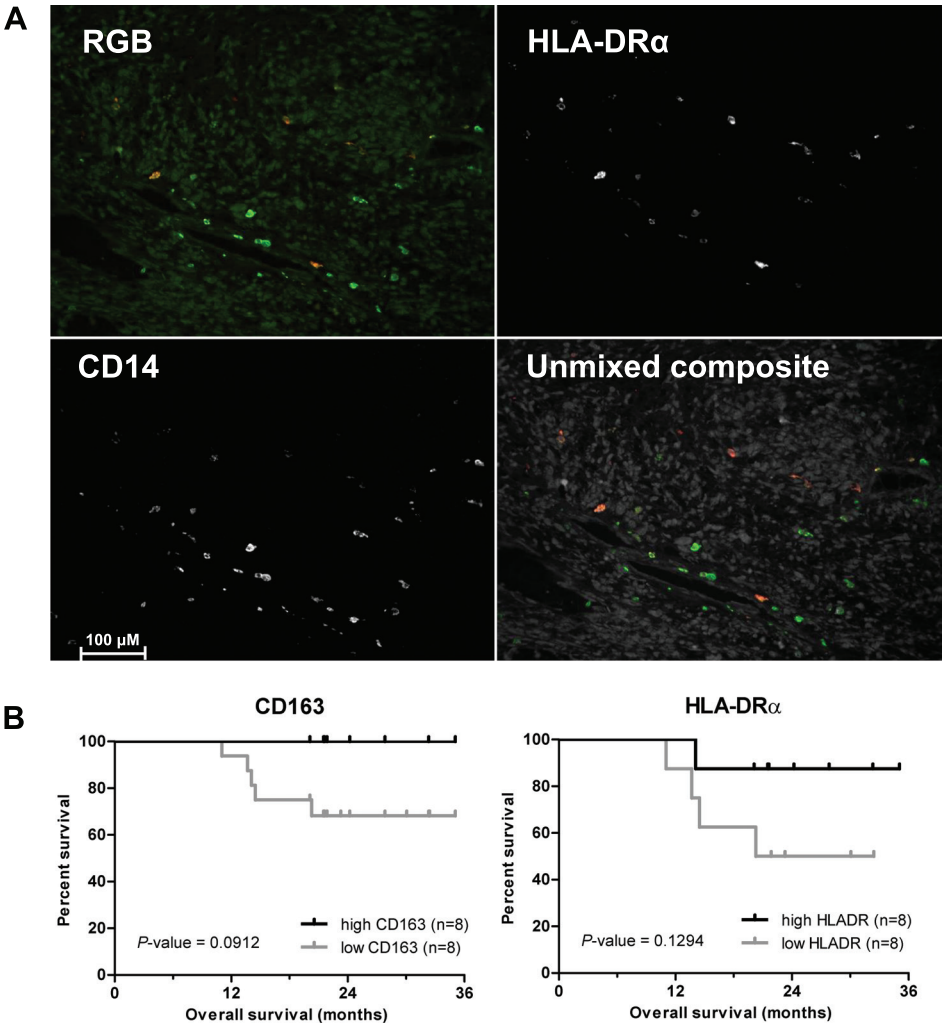


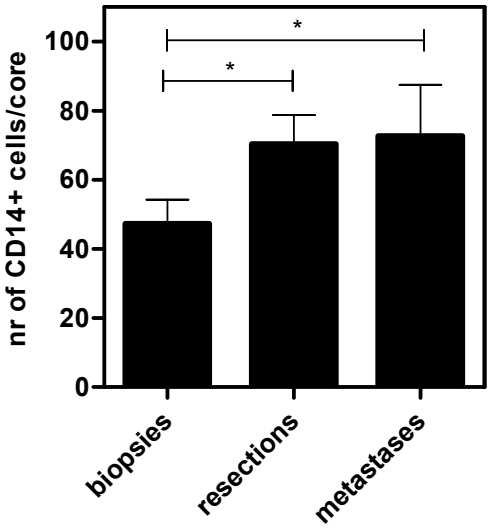
Suppl. Fig. 4.1 Gene expression levels of *CD14*, *HLA-DRA*, *CLEC5A*, and *FCGR2A* as obtained using genome-wide expression profiling correlated well with gene expression levels as determined using RT-qPCR.



Suppl. Fig. 4.2 High levels of *HLA-DRA*, *CLEC5A* and *FCGR2A* as determined using genome-wide expression profiling was associated with significantly improved metastases free survival. *P*-values obtained using logrank method, cut-off at the median (cohort 1).



Suppl. Fig. 4.3 **a**, osteosarcoma samples are infiltrated with CD14 and HLA-DRα single and double positive macrophages. Spectral imaging was used to reduce auto-fluorescence of osteosarcoma cells. In the composite image, CD14 positive cells are represented in green, HLA-DRα positive cells are represented in red, and CD14/HLA-DRα double positive cells are represented in yellow. Background auto-fluorescence of tumor cells is represented in grey. **b**, in 16 patients treated according to the EURAMOS-1 protocol (cohort 3), there was a trend for high macrophage infiltration as determined by immunofluorescent HLA-DRα and CD163 to be associated with improved overall survival. *P*-values obtained using logrank test, cut-off at the median.



Suppl. Fig. 4.4 In post-chemotherapy samples, macrophage numbers increased (post-chemotherapy resections of the primary tumor *vs.* pre-treatment diagnostic biopsies, cohort 2). Kruskal-Wallis test *P*-value = 0.0094, Dunn's post-test *P*-value <0.05 noted as *.

Suppl. Table 4.1 Clinicopathological data of osteosarcoma samples and patient characteristics. Treatment regimens containing high-dose methotrexate (M), doxorubicin (A), cisplatin (P) and/or ifosfamide (I) were used.

Patient characteristics	Cohort 1	Cohort 2	Cohort 3
Samples used for:	Microarray analysis	Tissue microarray: CD14 and CD31 staining	Immunofluorescent staining of CD14, CD163, HLA-DRα
Total nr of samples	53 (of 53 patients)	145 (of 88 patients)	29 (of 20 patients)
pre-treatment biopsies of primary tumor	53 (of 53 patients)	73 (of 73 patients)	16 (of 16 patients)
post-chemotherapy resections of primary tumor	0	45 (of 45 patients)	13 (of 13 patients)
metastatic lesions	0	24 (of 15 patients)	0
Year of diagnosis	1986-2006	1984-2003	2007-2008
Institution			
LUMC, Netherlands	27	all	all
IOR, Italy	7		
LOH, Sweden	2		
Radiumhospitalet, Norway	1		
WWUM, Germany	16		
Treatment regimens	MAPI, MAP, PIA, AP	AP, PIA	MAP
Location of primary tumor	n (%)	n (%)	n (%)
Femur	27 (50.1)	45 (51.1)	18(62.1)
Tibia/fibula	17 (32.1)	31 (35.2)	6 (20.7)
Humerus	8 (15.1)	10 (11.4)	5 (17.2)
Axial skeleton	0 (0)	1 (1.1)	0 (0)
Hand	0 (0)	1 (1.1)	0 (0)
Unknown/ other	1 (1.9)	0 (0)	1 (3.4)
Histological subtype			
Conventional osteosarcoma			
- osteoblastic	32 (60.4)	59 (67.0)	19 (65.5)
- chondroblastic	6 (11.3)	9 (10.2)	3 (10.3)
- fibroblastic	5 (9.4)	3 (3.4)	1 (3.4)
- unusual	7 (13.2)	8 (9.1)	2 (6.9)
Telangiectatic	3 (5.7)	6 (6.8)	4 (13.8)
High grade surface osteosarcoma	0 (0)	1 (1.1)	0 (0)
Small cell osteosarcoma	0 (0)	2 (2.3)	0 (0)
Histological response to pre-operative chemotherapy in the primary tumor			
Unknown	6 (11.3)	12 (13.6)	1 (3.4)
Poor response	29 (54.7)	48 (54.5)	15 (51.7)
Good response	18 (34.0)	28 (31.8)	13 (44.8)
Sex			
Male	33 (62.3)	47 (53.4)	14 (48.3)

Suppl. Table 4.2 RT-qPCR primer sequences. These sequences have been submitted to the Real Time PCR Primer and Probe Database (http://medgen.ugent.be/rtprimerdb/). All PCR products were validated by sequencing.

Gene symbol	Product size (bp)	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
CD14	198	GCCGCTGTGTAGGAAAGAAG	AGGTTCGGAGAAGTTGCAGA
CLEC5A	128	GGCGTTGGATCAACAACTCT	GATCCTGCGGTAGCTGATGT
FCGR2A	80	TATGTCCCAGAAACCTGTGG	GGGAGCAGCTTGACTGTCTG
HLA-DRA	141	TGTAAGGCACATGGAGGTGA	ATAGGGCTGGAAAATGCTGA

Suppl. Table 4.3 Antibodies and conditions used for immunohistochemistry and immunofluorescence.

Antibody	Clone/ catalogue nr	Company	Methods
CD31	Ab-1, clone JC/70A	Neomarkers Fremont, CA, USA	Antigen retrieval (AR): 1 mM EDTA solution (pH 8.0). Secondary antibody (2nd ab): Envision Horse Radish Peroxidase (HRP) anti-mouse (Dako), chromogen DAB+ (Dako, K3468)
CD14	Ab-2, clone 7	Neomarkers	AR: 10mM Tris/ 1 mM EDTA (pH 9.0). 2nd ab: Envision anti-mouse HRP (Dako) followed by DAB+; Alexa Fluor-488 goat anti-mouse IgG2a (Invitrogen)
CD163	NCL-CD163	Novocastra, Newcastle Upon Tyne, England	AR: 10mM Tris/ 1 mM EDTA (pH 9.0). 2nd ab: Envision anti-mouse HRP (Dako) followed by DAB+; Goat-anti-mouse alkaline phosphatase (AP) (Dako, nr) followed by Vector Blue (Vector Labs); Alexa Fluor-647 goat anti-mouse IgG1 (Invitrogen)
HLA-DRα	TAL.1B5	Dako	AR: 10mM Tris/ 1 mM EDTA (pH 9.0).2nd ab: Envision anti-mouse HRP (Dako) followed by DAB+; Goat-anti-mouse (AP) (Dako) followed by Vector Blue; Alexa Fluor-647 goat anti-mouse IgG1 (Invitrogen).

Suppl. Table 4.4 Differentially expressed genes and detailed descriptions including references. Probes with a positive log fold change (logFC) are higher in patients without metastases as compared to patients with metastases within five years. Adjusted *P*-value (adj*P*val) <0.05 determined to be significant.

logFC	adjPVal	Symbol	Description	Keywords	References
0.81	0.0035	*FCGR2B*	IgG FcR with a tyrosine-based inhibitory motif expressed on B lymphocytes, monocytes, neutrophils and myeloid dendritic cells.	Fc receptor, B lymphocytes, monocytes, neutrophils, myeloid dendritic cells	[250]
0.89	0.0038	*CLEC5A*	C-type lectin which binds Dengue virus. CLEC5A is highly expressed in murine neutrophils and macrophages. CLEC5a has a role in osteoclastogenesis.	C-type lectin, pattern recognition receptor, macrophages, neutrophils, osteoclasts, dengue virus	[9;39;106]
1.51	0.0083	*ALOX5AP*	This gene encodes a protein which, with 5-lipoxygenase, is required for leukotriene synthesis. Leukotrienes are arachidonic acid metabolites which have been implicated in various types of inflammatory responses and mediate production of endogenous PPAR-γ-ligands.	Arachidonic acid lipoxygenase, leukotrienes, PPAR- γ ligand production	[refseq] [267]
0.17	0.0110	*RNASE3*	Eosinophil cationic protein expressed mainly by eosinophils with antiviral, antibacterial and cytotoxic properties. Also produced by monocytes.	Eosinophils, cytotoxicity, monocytes	[36;271]
0.59	0.0171	*MSR1*	This gene encodes the class A macrophage scavenger receptors, which include three different types generated by alternative splicing. These receptors are macrophage-specific phagocytic pattern recognition receptors.	Scavenger receptor, pattern recognition, macrophages, phagocytosis	[refseq] [28]
1.12	0.0171	*FCGR2A*	IgG Fc receptor involved in phagocytosis by macrophages and neutrophils	Fc receptor, macrophages, neutrophils, phagocytosis	[refseq]
1.09	0.0173	*SPINT2*	Serine peptidase inhibitor, Kunitz type, 2. The protein inhibits HGF activator which prevents the formation of active hepatocyte growth factor. This gene is a putative tumor suppressor, and mutations in this gene result in congenital sodium diarrhea.	Tumor suppressor, hepatocyte growth factor inhibition	[refseq]
1.22	0.0173	*FGL2*	Fibrinogen-like 2 has immunosuppressive properties and binds FCGR2B and FCGR3	Immunosuppression, Fc receptor signaling	[147]
0.33	0.0173	*OGFRL1*	Opioid growth factor receptor-like 1 is related to OGF, which is a negative regulator of cell proliferation and tissue organization in a variety of processes.	Cell proliferation	[refseq]
0.63	0.0173	*CSF2RA*	The protein encoded by this gene is the alpha subunit of the heterodimeric receptor for colony stimulating factor 2, a cytokine which controls the production, differentiation, and function of granulocytes and macrophages.	Cytokine receptor, granulocytes, macrophages	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.79	0.0173	*CXCL16*	C-X-C motif ligand 16. Expression of CXCL16 by renal and colon cancer correlates with good prognosis. Associated with atherosclerosis. Chemo-attractant of T cells and macrophages.	Chemokine, macrophages, cancer, T cell	[91;101;162]
1.09	0.0173	*HLA-DOA*	Part of HLA class II alpa chain paralogues found in lysosomes in B cells. Regulates HLA-DM-mediated peptide loading on MHC class II molecules	HLA class II, B cells	[refseq]
1.23	0.0173	*HLA-DMB*	Part of HLA class II beta chain paralogues located in intracellular vesicles. DM plays a central role in the peptide loading of MHC class II molecules by helping to release the CLIP (class II-associated invariant chain peptide) molecule from the peptide binding site. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
1.14	0.0173	*CD86*	This protein is expressed by antigen-presenting cells (APC: B lymphocytes, dendritic cells, macrophages)., and it is the ligand for two proteins at the cell surface of T cells, CD28 antigen and cytotoxic T-lymphocyte-associated protein 4. Binding of this protein with CD28 antigen is a costimulatory signal for activation of the T-cell. Binding of this protein with cytotoxic T-lymphocyte-associated protein 4 negatively regulates T-cell activation and diminishes the immune response.	B cells, dendritic cells, macrophages, co-stimulation of T cells	[refseq]
0.73	0.0173	*VMO1*	Vitelline membrane outer layer 1 homolog	Unknown	
0.67	0.0173		AGENCOURT_10520654 NIH_MGC_128 Homo sapiens cDNA clone IMAGE:6702588 5, mRNA sequence	Unknown	
1.32	0.0173	*HLA-DMA*	Part of HLA class II beta chain paralogues located in intracellular vesicles. DM plays a central role in the peptide loading of MHC class II molecules by helping to release the CLIP (class II-associated invariant chain peptide) molecule from the peptide binding site. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
0.11	0.0173		UI-H-EZ1-bbe-e-05-0-UI.s1 NCI_CGAP_Ch2 Homo sapiens cDNA clone UI-H-EZ1-bbe-e-05-0-UI 3, mRNA sequence	Unknown	
0.64	0.0182	*NLRP3*	NLRP3 is a NOD-like receptor, and is a member of the NALP3 inflammasome complex. The inflammasome is a multiprotein complexe that can activates caspase-1 and ultimately lead to the processing and secretion of interleukin (IL)-1beta, IL-18 and IL-33. NLRP3 interacts with the apoptosis-associated speck-like protein PYCARD/ASC, which contains a caspase recruitment domain.	NOD-like receptor, inflammasome, monocyte, macrophage	[74;82]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.63	0.0182	*MSR1*	This gene encodes the class A macrophage scavenger receptors, which include three different types generated by alternative splicing. These receptors are macrophage-specific phagocytic pattern recognition receptors.	Scavenger receptor, pattern recognition, macrophages, phagocytosis	[refseq] [28]
0.61	0.0186	*PEA15*	PEA15 is a death effector domain (DED)-containing protein predominantly expressed in the central nervous system, particularly in astrocytes. Ubiquitously expressed, implicated in a number of fundamental cellular functions, including apoptosis, proliferation, and glucose metabolism. PED/PEA-15 is an endogenous substrate for protein kinase C (PKC), calcium/calmodulin-dependent protein kinase II (CAM kinase II), and Akt.	Apoptosis, proliferation, glucose metabolism, PKC, CAM kinase II, AKT.	[refeq] [68]
0.77	0.0187	*PKIB*	The protein encoded by this gene is a member of the cAMP-dependent protein kinase inhibitor family. PKIB and PKA-C kinase can have critical functions of aggressive phenotype of prostate cancer cells through Akt phosphorylation.	PKC, AKT, kinase inhibitor	[refseq] [45]
0.4	0.0187	*SYTL3*	Synaptotagmin-like 3, function unknown	Unknown	[refseq]
0.44	0.0187	*CUGBP2*	CUG triplet repeat, RNA binding protein 2, members of this protein family regulate pre-mRNA alternative splicing and may also be involved in mRNA editing, and translation.	RNA binding protein, alternative splicing	[refseq]
0.69	0.0215	*CSF2RA*	The protein encoded by this gene is the alpha subunit of the heterodimeric receptor for colony stimulating factor 2, a cytokine which controls the production, differentiation, and function of granulocytes and macrophages.	Cytokine receptor, granulocytes, macrophages	[refseq]
1.84	0.0215	*LYZ*	Lysozyme cleaves bacterial cell wall peptidoglycan, resulting in components that can bind the NOD receptor.	Bacterial wall peptidoglycan, NOD	[refseq], [82]
1.1	0.0215	*FPRL2*	Formyl peptide receptors sense formyl peptides in bacteria and mitochondria. These receptors are expressed on phagocytic cells.	Formyl peptide receptor, pattern recognition receptor, phagocytosis	[171]
0.83	0.0217	*FCGR2B*	IgG FcR with a tyrosine-based inhibitory motif expressed on B lymphocytes, monocytes, neutrophils and myeloid dendritic cells.	Fc receptor, B lymphocytes, monocytes, neutrophils, myeloid dendritic cells	[250]
0.69	0.0246	*PIK3IP1*	phosphoinositide-3-kinase interacting protein 1 is a negative regulator of PI3K and suppresses the development of hepatocellular carcinoma	PI3K, AKT, tumor suppressor	[99]
1.49	0.0246	*HLA-DRA*	HLA-DRA is one of the HLA class II alpha chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.85	0.0276	*HLA-DPB1*	HLA-DPB1 belongs to the HLA class II beta chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
0.32	0.0276	*KCNK13*	This gene encodes one of the members of the superfamily of potassium channel proteins containing two pore-forming domains. The product of this gene is an open channel that can be stimulated by arachidonic acid.	Potassium channel, arachinodic acid	[refseq]
0.47	0.0276	*LRRC25*	Leucine rich repeat containing 25, high expressed in plasmacytoid dendritic cells and granulocytes.	Dendritic cells, granulocytes	[216]
1.49	0.0276	*CD74*	Major histocompatibility complex, class II invariant chain. May also interact with CXCR4 to bind macrophage migration inhibitory factor.	HLA class II, B cell, dendritic cells, macrophages, macrophage migration, CXCR4	[184;232]
0.71	0.0276	*CTSS*	Cathepsin S is a lysosomal cysteine proteinase that may participate in the degradation of antigenic proteins to peptides for presentation on MHC class II molecules.	Cathepsin, HLA class II, B cells, dendritic cells, macrophages	[refseq]
0.49	0.0276	*PFKFB2*	The protein encoded by this gene is involved in both the synthesis and degradation of fructose-2,6-bisphosphate, a regulatory molecule that controls glycolysis in eukaryotes.	Glycolysis, glucose metabolism	[refseq]
1.47	0.0276	*HLA-DPA1*	HLA-DPA1 is one of the HLA class II alpha chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
0.88	0.0276	*CD37*	Member of the transmembrane 4 superfamily. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. Expressed by immune cells, including macrophages and osteoclasts.	Tetraspanin, B cells, macrophages, osteoclasts.	[refseq], [11]
0.55	0.0276	*PILRA*	Control of cell signaling via SHP-1 is thought to occur through a balance between PILRalpha-mediated inhibition and PILRbeta-mediated activation.	Cell signalling, SHP-1	[refseq]
0.81	0.0276	*TLR7*	TLR7 recognizes single stranded RNA. TLR7 is expressed in a variety of different cell types of the immune system such as DCs, B cells, monocytes, NK cells and T cells.	Toll-like receptor, pattern recognition receptor, dendritic cells, B cells, monocytes, NK cells, T cells, ssRNA	[16]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
1.15	0.0276	*FCGR2A*	IgG Fc receptor involved in phagocytosis by macrophages and neutrophils	Fc receptor, macrophages, neutrophils, phagocytosis	[refseq]
0.68	0.0276	*TLR6*	This receptor functionally interacts with toll-like receptor 2 to mediate cellular response to bacterial lipoproteins.	Toll-like receptor, pattern recognition receptor, bacterial lipoproteins	[refseq]
0.55	0.0276	*TM6SF1*	Transmembrane 6 superfamily member 1, expressed in hematopoietic cells and testis, function unknown.	Hematopoietic cells	[37]
0.56	0.0276	*CLEC12A*	The C-type lectin encoded by this gene is a negative regulator of granulocyte and monocyte function.	C-type lectin, pattern recognition receptor, bacteria, monocytes, granulocytes	[refseq]
0.36	0.0276	*LY9*	LY9 belongs to the SLAM family of immunomodulatory receptors and interacts with the adaptor molecule SAP. SLAM family of receptors and SAP family of adaptors play critical roles in lymphocyte development, differentiation, and acquisition of effector functions.	SLAM, SAP, lymphocyte development, immune cell effector function	[refseq], [152]
0.36	0.0276	*SLC2A9*	This gene encodes a member of the SLC2A facilitative glucose transporter family. Members of this family play a significant role in maintaining glucose homeostasis. The encoded protein may play a role in the development and survival of chondrocytes in cartilage matrices.	Glucose transport, chondrocyte development	[refseq]
1.22	0.0276	*AIF1*	This gene is induced by cytokines and interferon. Expressed by activated macrophages and regulates endothelial cell activation, signal transduction, and vasculogenesis.	Cytokines, interferon, endothelial cells, macrophages	[refseq], [257;258]
0.79	0.0277	*TNFRSF1B*	The protein encoded by this gene is a member of the TNF-receptor superfamily. This protein and TNF-receptor 1 form a heterocomplex that mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2, which possess E3 ubiquitin ligase activity. The function of IAPs in TNF-receptor signalling is unknown, however, c-IAP1 is thought to potentiate TNF-induced apoptosis by the ubiquitination and degradation of TNF-receptor-associated factor 2, which mediates anti-apoptotic signals.	TNF-receptor superfamily, apoptosis.	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.59	0.0280	*LITAF*	Lipopolysaccharide is a potent stimulator of monocytes and macrophages, causing secretion of tumor necrosis factor-alpha (TNF-alpha) and other inflammatory mediators. This gene encodes lipopolysaccharide-induced TNF-alpha factor, which is a DNA-binding protein and can mediate the TNF-alpha expression by direct binding to the promoter region of the TNF-alpha gene. The transcription of this gene is induced by tumor suppresor p53 and has been implicated in the p53-induced apoptotic pathway. Mutations in this gene cause Charcot-Marie-Tooth disease type 1C (CMT1C) and may be involved in the carcinogenesis of extramammary Paget's disease (EMPD).	Pattern recognition signalling, monocytes, macrophages, TNF-alpha, p53-induced apoptotic pathway	[refseq]
0.82	0.0289	*ARHGAP30*	Rho GTPase, They play a critical role in muscle differentiation. The protein encoded by this gene binds GTP and is a member of the small GTPase superfamily. It is involved in endosome dynamics and reorganization of the actin cytoskeleton, and it may coordinate membrane transport with the function of the cytoskeleton.	Rho GTPase, cytoskeleton.	[refseq]
1.6	0.0303	*HLA-DRB4*	HLA-DRB4 belongs to the HLA class II beta chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
1	0.0322	*IRF8*	The IRF family proteins bind to the IFN-stimulated response element (ISRE) and regulate expression of genes stimulated by type I IFNs, namely IFN-alpha and IFN-beta. Expression of IRF8 is inducible by IFN-gamma. Its target genes are IL-12 and IL-18.	Interferon	[refseq]
0.17	0.0322	*GPX1*	This gene encodes a member of the glutathione peroxidase family. Glutathione peroxidase functions in the detoxification of hydrogen peroxide, and is one of the most important antioxidant enzymes in humans.	Gluthation peroxidase, antioxidant	[refseq]
0.46	0.0322	*HMHA1*	Rho-like GTPase-activating protein, minor histocompatibility antigen restricted to hematopoietic cells.	Minor histocompatibility antigen, hematopoietic cells, Rho-like GTPase-activating protein	[244]
0.1	0.0332	*OR4K15*	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell. The olfactory receptor proteins are members of a large family of G-protein-coupled receptors (GPCR) arising from single coding-exon genes.	G-protein-coupled receptors, olfactory receptor	[refseq]
0.62	0.0332	*RGS10*	Regulator of G protein signaling (RGS) family members are regulatory molecules that act as GTPase activating proteins (GAPs) for G alpha subunits of heterotrimeric G proteins. RGS10A is a key component in the RANKL-evoked signaling pathway for osteoclast differentiation.	G-protein signalling, GTPase activating proteins, osteoclast differentiation	[refseq], [283]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.38	0.0332	*CYP2S1*	This gene encodes a member of the cytochrome P450 superfamily of enzymes. CYP2S1 is inducible by dioxin, the induction being mediated by the Aryl Hydrocarbon Receptor (AHR) and Aryl Hydrocarbon Nuclear Translocator (ARNT). The observed ubiquitous tissue distribution, as well as the expression of CYP2S1 throughout embryogenesis suggest that CYP2S1 is likely to metabolize important endogenous substrates; thus far, retinoic acid has been identified.	Cytochrome P450, aryl hydrocarbon signalling	[refseq], [226]
-0.23	0.0332	*NHN1*	conserved nuclear protein NHN1	Unknown	[refseq]
0.96	0.0332	*SLCO2B1*	solute carrier organic anion transporter family, member 2B1. Possible drug transporter.	Solute transporter	[refseq]
1.31	0.0341	*CD14*	CD14 is a surface protein preferentially expressed on monocytes/macrophages, and associates with TLR4 in lipopolysaccaride binding.	Monocytes, macrophages, lipopolysaccharide, pattern recognition receptor	[refseq]
0.2	0.0347	*NAIP*	NOD-like receptor	NOD-like receptor, inflammasome, monocyte, macrophage	[82]
1.09	0.0347	*ITGB2*	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit). This gene encodes the integrin beta chain beta 2. A given chain may combine with multiple partners resulting in different integrins. For example, beta 2 combines with the alpha L chain to form the integrin LFA-1, and combines with the alpha M chain to form the integrin Mac-1. Integrins are known to participate in cell adhesion as well as cell-surface mediated signalling. Defects in this gene are the cause of leukocyte adhesion deficiency type I (LAD1).	Integrins, leukocytes, signalling	[refseq]
1.27	0.0347	*MS4A6A*	This gene encodes a member of the membrane-spanning 4A gene family. Members of this nascent protein family are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns among hematopoietic cells and nonlymphoid tissues.	Unknown	[refseq]
-0.75	0.0347	*HOXC4*	This gene belongs to the homeobox family of genes. The homeobox genes encode a highly conserved family of transcription factors that play an important role in morphogenesis in all multicellular organisms.	Homeobox gene, morphogenesis	[refseq]
1.6	0.0347	*HLA-DQA1*	HLA-DQA1 is one of the HLA class II alpha chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.39	0.0347	*IQGAP2*	This gene encodes a member of the IQGAP family. The protein contains three IQ domains, one calponin homology domain, one Ras-GAP domain and one WW domain. It interacts with components of the cytoskeleton, with cell adhesion molecules, and with several signaling molecules to regulate cell morphology and motility. Putative tumor suppressor.	Cytoskeleton, cell adhesion, signaling, tumor suppressor	[refseq], [279]
-0.2	0.0347	*MAP2K7*	The protein encoded by this gene is a dual specificity protein kinase that belongs to the MAP kinase kinase family. This kinase is involved in the signal transduction mediating the cell responses to proinflammatory cytokines, and environmental stresses. Expressed by macrophages and involved in osteoclastogenesis.	MAP kinase signal transduction, cytokine response, macrophages, osteoclasts	[refseq],[282]
-0.48	0.0350	*BRWD1*	This gene encodes a member of the WD repeat protein family. Members of this family are involved in a variety of cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation. This protein contains 2 bromodomains and multiple WD repeats, and the function of this protein is not known.	Unknown	[refseq]
1.2	0.0355	*HCST*	HCST (or DAP10) is a signaling molecule which associates with C-type lectins, such as CLEC5A.	C-type lectin, pattern recognition signaling, osteoclasts.	[106]
0.91	0.0358	*DOCK2*	Dedicator of cytokinesis 2 (DOCK2) gene encodes a hematopoietic cell-specific CDM family protein that is indispensable for lymphocyte chemotaxis.	Hematopoietic cells, chemotaxis	[refseq]
0.44	0.0358	*ADAM28*	This gene encodes a member of the ADAM (a disintegrin and metalloprotease domain) family. Members of this family are membrane-anchored proteins structurally related to snake venom disintegrins, and have been implicated in a variety of biological processes involving cell-cell and cell-matrix interactions, including fertilization, muscle development, and neurogenesis. The protein encoded by this gene is a lymphocyte-expressed ADAM protein.	Cell-cell interaction, lymphocytes	[refseq]
0.77	0.0361	*LOC387841*	ribosomal protein L13a pseudogene 20	Unknown	[refseq]
1.06	0.0363	*HCLS1*	Hematopoietic cell-specific Lyn substrate 1, actin-regulatory adaptor protein at the immune synapse	Hematopoietic cells, src family kinase substrate, cytoskeleton	[85]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.9	0.0371	*SLC11A1*	Natural resistance-associated macrophage protein 1. This gene is a member of the solute carrier family 11 (proton-coupled divalent metal ion transporters) family and encodes a multi-pass membrane protein. The protein functions as a divalent transition metal (iron and manganese) transporter involved in iron metabolism and host resistance to certain pathogens. Mutations in this gene have been associated with susceptibility to infectious diseases such as tuberculosis and leprosy, and inflammatory diseases such as rheumatoid arthritis and Crohn disease.	Solute carrier, macrophages, tuberculosis	[refseq]
-0.08	0.0371	*LOC652140*	similar to DNA-directed RNA polymerase II largest subunit	Unknown	[refseq]
0.45	0.0371	*MARCH1*	Membrane-associated ring finger (C3HC4) 1 is a major regulator of HLA-DR traffic.	HLA class II, B cells, dendritic cells, macrophages	[55]
0.66	0.0374	*SEMA4A*	SEMA4A is a member of the semaphorin family of soluble and transmembrane proteins. Semaphorins are involved in guidance of axonal migration during neuronal development and in immune responses, particularly Th1 type responses through the receptor Tim-2.	Semaphorin, axonal migration, immunology, Th-1 response	[refseq], [187]
0.54	0.0374	*CD86*	This protein is expressed by antigen-presenting cells (APC: B lymphocytes, dendritic cells, macrophages) and it is the ligand for two proteins at the cell surface of T cells, CD28 antigen and cytotoxic T-lymphocyte-associated protein 4. Binding of this protein with CD28 antigen is a costimulatory signal for activation of the T-cell. Binding of this protein with cytotoxic T-lymphocyte-associated protein 4 negatively regulates T-cell activation and diminishes the immune response.	B cells, dendritic cells, macrophages, co-stimulation of T cells	[refseq]
0.92	0.0390	*LST1*	Higher expressed on CD16+ than CD16- monocytes. It is expressed in T cell, monocytic and macrophage cell lines, and it is substantially expressed in both primary human and murine dendritic cells (DCs) in culture.	Monocytes, macrophages, T cells, DCs	[6;189]
0.64	0.0398	*TLR8*	TLR8 recognizes nucleoside analogues. It is expressed in a variety of different cell types of the immune system such as DCs, B cells, monocytes, NK cells and T cells.	Toll-like receptor, pattern recognition receptor, dendritic cells, B cells, monocytes, NK cells, T cells, nucleoside analogues	[16]
0.57	0.0398	*BIN2*	The Bin2 gene is expressed predominantly in hematopoietic cells and is upregulated during differentiation of granulocytes.	Hematopoietic cells, granulocytes	[81]
0.83	0.0398	*TMEM149*	transmembrane protein 149	Unknown	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.16	0.0398	*MICALCL*	MICAL C-terminal like, function unknown, but MICAL has a role in semaphorin signaling	Unknown, possibly semaphorin signaling	[refseq], [127]
1.29	0.0398	*MS4A6A*	This gene encodes a member of the membrane-spanning 4A gene family. Members of this nascent protein family are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns among hematopoietic cells and nonlymphoid tissues.	Unknown	[refseq]
0.56	0.0405	*TLR4*	This receptor is most abundantly expressed in placenta, and in the myelomonocytic subpopulation of the leukocytes. It has been implicated in signal transduction events induced by lipopolysaccharide found in most gram-negative bacteria.	Toll-like receptor, placenta, monocytes, macrophages, lipopolysaccharide, pattern recognition receptor	[refseq]
1.01	0.0405	*GIMAP4*	This gene encodes a protein belonging to the GTP-binding superfamily and to the immuno-associated nucleotide (IAN) subfamily of nucleotide-binding proteins. Upregulated during Th1 differentiation.	GTP-binding protein, Th1 cells	[refseq], [67]
0.4	0.0415	*C12orf35*	chromosome 12 open reading frame 35	Unknown	[refseq]
0.26	0.0415	*CUGBP2*	CUG triplet repeat, RNA binding protein 2, members of this protein family regulate pre-mRNA alternative splicing and may also be involved in mRNA editing, and translation.	RNA binding protein, alternative splicing	[refseq]
0.55	0.0415	*MFNG*	This gene is a member of the fringe gene family which also includes radical and lunatic fringe genes. They all encode evolutionarily conserved secreted proteins that act in the Notch receptor pathway to demarcate boundaries during embryonic development. While their genomic structure is distinct from other glycosyltransferases, fringe proteins have a fucose-specific beta-1,3-N-acetylglucosaminyltransferase activity that leads to elongation of O-linked fucose residues on Notch, which alters Notch signaling.	Notch receptor pathway signaling. Gycosyltransferase.	[refseq]
0.21	0.0415	*NLRC4*	NOD-like receptor	NOD-like receptor, inflammasome, monocyte, macrophage	[82]
-0.29	0.0415	*MRPL4*	Mitochondrial ribosomal protein L4, 39S subunit. Mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion. Mitochondrial ribosomes (mitoribosomes) consist of a small 28S subunit and a large 39S subunit.	Mitochondrial ribosomal protein	[refseq]
0.65	0.0415	*PARVG*	Members of the parvin family, including PARVG, are actin-binding proteins associated with focal contacts. Essential for the establishment of cell polarity required for leukocyte migration.	Cytoskeleton, leukocyte migration	[refseq], [284]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.49	0.0415	*AOAH*	Acyloxyacyl hydrolase (AOAH) is a 2-subunit lipase which selectively hydrolyzes the secondary (acyloxyacyl-linked) fatty acyl chains from the lipid A region of bacterial endotoxins. AOAH may modulate host inflammatory responses to gram-negative bacterial invasion. Expressed by leukocytes.	Lipase, bacterial endotoxins, leukocytes.	[refseq]
0.45	0.0415	*PSCD1*	The protein encoded by this gene is a member of the PSCD family. Members of this family appear to mediate the regulation of protein sorting and membrane trafficking. This gene is highly expressed in natural killer and peripheral T cells, and regulates the adhesiveness of integrins at the plasma membrane of lymphocytes.	Natural killer cells, T cells, integrins	[refseq]
0.7	0.0415	*IL10RA*	The protein encoded by this gene is a receptor for interleukin 10. This protein is structurally related to interferon receptors. It has been shown to mediate the immunosuppressive signal of interleukin 10, and thus inhibits the synthesis of proinflammatory cytokines. This receptor is reported to promote survival of progenitor myeloid cells through the insulin receptor substrate-2/PI 3-kinase/AKT pathway. Activation of this receptor leads to tyrosine phosphorylation of JAK1 and TYK2 kinases.	Leukocytes, monocytes, macrophages, cytokines, PI 3-kinase/AKT pathway, immunosuppression	[refseq]
1.15	0.0415	*ALOX5*	This gene encodes a member of the lipoxygenase gene family and plays a dual role in the synthesis of leukotrienes from arachidonic acid. Leukotrienes are arachidonic acid metabolites which have been implicated in various types of inflammatory responses and mediate production of endogenous PPAR-γ-ligands. Mutations in the promoter region of this gene lead to a diminished response to antileukotriene drugs used in the treatment of asthma and may also be associated with atherosclerosis and several cancers. .	Arachidonic acid lipoxygenase, leukotrienes, PPAR- γ ligand production, astma, cancer	[refseq] [267]
0.31	0.0415	*PAOX*	FAD-dependent polyamine oxidase is one of the key enzymes in the catabolism of polyamines spermidine and spermine.	Polyamine oxidase	[111]
0.49	0.0415	*APPL2*	APPL is an effector of the small GTPase Rab5, a key regulator of early steps of endocytosis. In addition, APPL proteins exert their stimulatory effects on beta-catenin/TCF-dependent transcription by decreasing the activity of a Reptin-containing repressive complex.	Endocytosis, beta-catenin/ TCF-dependent transcription	[212]
1.04	0.0415	*FHL2*	Four and a half LIM domains 2, expressed by heart muscle, cancer cells and osteoblasts. FHL2 transcript levels increased threefold during differentiation of mouse bone marrow cells into osteoblasts. Also influences Wnt signalling.	Osteoblasts, Wnt signalling, heart muscle	[115]
0.48	0.0415	*DPEP2*	DPEP2 belongs to the membrane-bound dipeptidase family. These enzymes hydrolyze a variety of dipeptides, including leukotriene D4, the beta-lactam ring of some antibiotics, and cystinyl-bis-glycine (cys-bis-gly) formed during glutathione degradation.	Dipeptidase	[refseq]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
0.87	0.0415	*PTPN6*	Protein tyrosine phosphatase, non-receptor type 6. This PTP is expressed primarily in hematopoietic cells, and functions as an important regulator of multiple signaling pathways in hematopoietic cells. Amongst others, associates with phosphorylated immunoreceptor tyrosine-based activation motif of Fc gamma RIIa to modulate signaling events in myeloid cells.	Hematopoietic cells, Fc receptor signaling, myeloid cells	[refseq] [79]
1.3	0.0415	*C1QA*	Complement component 1, q subcomponent, A chain. Deficiency is associated with lupus erythematosus and glomerulonephritis. Expressed by monocytes and macrophages.	Complement, SLE, monocytes, macrophages	[refseq] [90]
0.3	0.0415	*MPEG1*	Macrophage expressed 1, may share a distant ancestry to perforin.	Macrophages.	[245]
0.94	0.0415	*HAVCR2*	Phagocytic receptor responsible for cross-presentation of dying cell-associated antigens, expressed on macrophages and DCs. Also expressed by Th1 cells.	Phagocytosis, macrophages, DCs, Th1 cells, apoptosis	[192]
1.11	0.0415	*AMICA1*	Junctional adhesion molecule-like, expressed on hematopoietic cells, mainly monocytes and granulocytes	Adhesion, monocyte, granulocyte	[185]
0.71	0.0415	*IFNGR1*	This gene (IFNGR1) encodes the ligand-binding chain (alpha) of the gamma interferon receptor. Human interferon-gamma receptor is a heterodimer of IFNGR1 and IFNGR2.	Interferon gamma receptor	[refseq], [103]
-0.41	0.0421	*NSUN5*	This gene encodes a member of the evolutionarily conserved NOL1/NOP2/Sun domain family. The encoded protein may function as a DNA methyltransferase in the nucleus.	DNA methyltransferase	[refseq]
-0.08	0.0429	*DNAI2*	Dynein, axonemal, intermediate chain 2 is essential for outer dynein arm assembly. DNAI2 mutations result in primary ciliary dyskinesia and randomization of left/right body asymmetry.	Dynein, cytoskeleton, ciliary dyskinesia	[149]
0.1	0.0434		ns42b07.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:1186261 3, mRNA sequence	Unknown	
0.41	0.0438	*VAV1*	The protein encoded by this proto-oncogene is a member of the Dbl family of guanine nucleotide exchange factors (GEF) for the Rho family of GTP binding proteins. The protein is important in hematopoiesis, playing a role in T-cell and B-cell development and activation. This particular GEF has been identified as the specific binding partner of Nef proteins from HIV-1. Coexpression and binding of these partners initiates profound morphological changes, cytoskeletal rearrangements and the JNK/SAPK signaling cascade, leading to increased levels of viral transcription and replication.	Oncogene, hematopoietic cells, JNK/SAPK signaling	[refseq]
0.55	0.0438	*MERTK*	C-mer proto-oncogene tyrosine kinase is a receptor involved in phagocytosis of (pro-) apoptotic cells by macrophages. Mutations in this gene have been associated with disruption of the retinal pigment epithelium (RPE) phagocytosis pathway and onset of autosomal recessive retinitis pigmentosa.	Macrophages, apoptosis, phagocytosis, retinitis pigmentosa	[refseq] [265]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
1	0.0438	*LY86*	Myeloid differentiation-1 associates with the toll-like protein RP105 in B cells and is downregulated on monocytes following BCG infection.	Toll signaling, B cells, monocytes	[17;180]
0.61	0.0438	*MYO1G*	Myosin IG, also known as minor histocompatibility antigen HA-2, expressed by hematopoietic cells.	Hematopoietic cells, minor histocompatibility antigen	[197]
0.19	0.0443	*RPGRIP1*	This gene encodes a photoreceptor protein that interacts with retinitis pigmentosa GTPase regulator protein and is a key component of cone and rod photoreceptor cells.	Photoreceptor protein, autosomal recessive congenital blindness	[refseq]
0.42	0.0446	*ASGR1*	The asialoglycoprotein receptor binds to desialylated (galactosyl-terminal) plasma glycoproteins, removing them from the circulation. It transports these glycoproteins via a series of membrane vesicles and tubules to an acidic-sorting organelle where the receptor and ligand dissociate. Then the receptor is recycled back to the cell surface and the ligand is transported to the lysosomes for degradation. Expressed by hepatocytes and macrophages.	Glycoproteins, hepatic cells, endocytosis, macrophages	[refseq], [241]
0.33	0.0446	*PLEKHA7*	Part of the protein-complex in the zonula adherens, a specialized cadherin-based structure found at the contacts between epithelial cells where microtubules attach.	Zonula adherens, cytoskeleton.	[166]
0.83	0.0446	*IL1B*	This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE).	Caspase activation, inflammasome, cytokine, inflammation, osteoclast	[refseq]
0.07	0.0455		AB076959 Human vestibular cDNA library Homo sapiens cDNA clone 415V5-6-11, mRNA sequence	Unknown	
0.06	0.0463	*C20orf174*		Unknown	
-0.06	0.0469	*LOC649978*		Unknown	
-0.95	0.0473	*GHR*	This gene encodes a protein that is a transmembrane receptor for growth hormone. Binding of growth hormone to the receptor leads to receptor dimerization and the activation of an intra- and intercellular signal transduction pathway leading to growth.	Growth hormone receptor	[refseq]
-0.15	0.0473		UI-H-BI1-aem-a-04-0-UI.s1 NCI_CGAP_Sub3 Homo sapiens cDNA clone IMAGE:2719758 3, mRNA sequence	Unknown	
0.66	0.0475	*P2RY13*	The product of this gene belongs to the family of G-protein coupled receptors. This receptor is activated by ADP and is a high-affinity receptor for HDL apolipoprotein A-I (apoA-I) on human hepatocytes.	G-protein coupled receptor, HDL apolipoprotein, human hepatocytes.	[refseq], [108]

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
1	0.0475	*CECR1*	This gene encodes a member of a subfamily of the adenosine deaminase protein family. The encoded protein may act as a growth factor and have adenosine deaminase activity. It may be active in sites of inflammation during hypoxia and in areas of tumour growth.	Adenosine deaminase, inflammation, hypoxia, tumor growth	[refseq], [285]
1	0.0475	*C1orf162*		Unknown	
0.89	0.0475	*PYCARD*	The PYD and CARD domains are members of the six-helix bundle death domain-fold superfamily that mediates assembly of large signaling complexes in the inflammatory and apoptotic signaling pathways via the activation of caspase.	Caspase activation, inflammasome, monocyte, macrophage	[refseq], [74]
0.54	0.0483	*TMBIM4*	Transmembrane BAX inhibitor motif containing 4, may participate in cell death regulation by interacting with proteins of Bcl-2 family, thus promoting tumor metastasis.	BAX, BCL-2, apoptosis, metastasis	[286]
0.36	0.0485	*SLC4A7*	Solute carrier family 4, sodium bicarbonate cotransporter, member 7. Involved in bicarbonate transport, expressed by osteoclasts, heart, skeletal muscle, and kidney in which it plays an important role in HCO(3)(-) metabolism.	Bicarbonate transporter, osteoclasts, heart, msucle, kidney.	[150;215]
0.49	0.0485	*NECAP2*	This gene likely encodes a member of the adaptin-ear-binding coat-associated protein family. Studies of a similar protein in rat suggest a role in clathrin-mediated endocytosis.	Endocytosis	[refseq]
0.91	0.0499	*WAS*	The Wiskott-Aldrich syndrome (WAS) family of proteins share similar domain structure, and are involved in transduction of signals from receptors on the cell surface to the actin cytoskeleton. The presence of a number of different motifs suggests that they are regulated by a number of different stimuli, and interact with multiple proteins. Recent studies have demonstrated that these proteins, directly or indirectly, associate with the small GTPase, Cdc42, known to regulate formation of actin filaments, and the cytoskeletal organizing complex, Arp2/3. The WAS gene product is a cytoplasmic protein, expressed exclusively in hematopoietic cells.	Arp2/3, cytoskeleton, hematopoietic cells	[refseq]
0.45	0.0499	*AADACL1*	Arylacetamide deacetylase-like 1, also known as neutral cholesterol ester hydrolase 1 is expressed by macrophages in atherosclerotic plaques. Also highly expressed in aggressive cancer cells.	Cholesterol ester hydrolase, lipid metabolism, macrophages, cancer	[40;196]
0.08	0.0499		BX112750 Soares placenta Nb2HP Homo sapiens cDNA clone IMAGp998K15220, mRNA sequence	Unknown	
0.07	0.0499		ze37g04.r1 Soares retina N2b4HR Homo sapiens cDNA clone IMAGE:361206 5, mRNA sequence	Unknown	

Suppl. Table 4.4 *Continued*

logFC	adjPVal	Symbol	Description	Keywords	References
-0.27	0.0499	*BCCIP*	BRCA2 and CDKN1A interacting protein, This gene product was isolated on the basis of its interaction with BRCA2 and p21 proteins. It is an evolutionarily conserved nuclear protein with multiple interacting domains. The N-terminal half shares moderate homology with regions of calmodulin and M-calpain, suggesting that it may also bind calcium. Functional studies indicate that this protein may be an important cofactor for BRCA2 in tumor suppression, and a modulator of CDK2 kinase activity via p21. This protein has also been implicated in the regulation of BRCA2 and RAD51 nuclear focus formation, double-strand break-induced homologous recombination, and cell cycle progression. BCCIP is essential for completion of cytokinesis.	BRCA2, p21, DNA damage response pathway, homologous recombination, cell cycle control, cytokinesis.	[refseq], [167]
-0.06	0.0499	*C3orf42*		Unknown	
0.65	0.0499	*LST1*	Higher expressed on CD16+ than CD16- monocytes. It is expressed in T cell, monocytic and macrophage cell lines, and it is substantially expressed in both primary human and murine dendritic cells (DCs) in culture.	Monocytes, macrophages, T cells, DCs	[6;189]
1.32	0.0499	*HLA-DQB1*	HLA-DQB1 belongs to the HLA class II beta chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells (APC: B lymphocytes, dendritic cells, macrophages).	HLA class II, B cells, dendritic cells, macrophages	[refseq]
-0.11	0.0499	*SLC24A4*	Potassium-dependent sodium/calcium exchangers, such as NCKX4, are thought to transport 1 intracellular calcium and 1 potassium ion in exchange for 4 extracellular sodium ions.	Sodium/potassium/calcium exchanger	[refseq]
0.09	0.0499	*GGN*	This gene is a germ cell-specific gene that encodes proteins that interact with POG (proliferation of germ cells). These proteins regulate the localization of POG and may play a role in spermatogenesis.	Germ cell-specific gene, spermatogenesis.	[refseq]