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ORIGINAL ARTICLE

Benefit of adjuvant interferon alfa-2b (IFN-α) therapy in melanoma patients with high serum MMP-8 levels

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Abstract Matrix metalloproteinases (MMPs) are important enzymes in tissue turnover and various inflammatory processes. In this study, it was evaluated whether serum MMP-8 can predict the response to adjuvant interferon alfa-2b (IFN-α) therapy in patients with operated high-risk cutaneous melanoma. Pre-treatment sera from 460 patients with stage IIB–IIIC melanoma were analyzed for MMP-8. The patients were randomized after surgery to adjuvant IFN-α for 12 or 24 months ($n = 313$) or observation only ($n = 147$). The median serum MMP-8 level was used to classify the patients into a low MMP-8 ($n = 232$) and a high MMP-8 ($n = 228$) group. In the high MMP-8 subgroup, IFN-α therapy significantly improved relapse-free survival (RFS). RFS was 36.8 months in patients with high MMP-8 levels receiving IFN-α therapy, whereas RFS for those with high MMP-8 levels with observation only was 10.6 months ($P = 0.027$). Median overall survival for patients with high MMP-8 and observation only was 36.7 versus 71.7 months in those receiving IFN-α ($P = 0.13$). In a multivariate model, IFN-α therapy was a significant predictor of favorable RFS (HR 0.74; 95 % CI 0.55–0.99; $P = 0.048$), after adjustment for pre-treatment MMP-8 (HR 1.17; 95 % CI 0.88–1.55; $P = 0.28$), gender (HR 1.16; 95 % CI 0.86–1.56; $P = 0.32$), age (HR 1.00; 95 % CI 1.00–1.02; $P = 0.12$), ulceration (HR 1.09; 95 % CI 0.81–1.46; $P = 0.58$), and the presence of node metastases (HR 1.36; 95 % CI 1.17–1.58; $P < 0.0001$). In conclusion, patients with high serum MMP-8 levels may benefit from

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adjuvant IFN-α therapy, but this observation should be further investigated.

Keywords Adjuvant interferon · Melanoma · MMP · Prognosis · Serum · Survival

Abbreviations

AJCC	American Joint Committee on Cancer
ANOVA	Analysis of variance
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
CI	Confidence interval
CV	Coefficent of variation
ECOG	Eastern cooperative oncology group
HNSCC	Head and neck squamous cell carcinomas
HR	Hazard ratio
IFMA	Immunofluorometric assay
IFN	Interferon
ISG	Interferon stimulated gene
KRAS	Kirsten rat sarcoma viral oncogene homolog
LDH	Lactate dehydrogenase
MIA	Melanoma-inhibiting activity
MMP	Matrix metalloproteinase
NRAS	Neuroblastoma RAS viral oncogene homolog
OS	Overall survival
RFS	Relapse-free survival

Introduction

Matrix metalloproteinases (MMPs) form a group of 25 structurally related but genetically distinct Zn2+-dependent endoproteases [1]. MMP-8 is a collagenase-2 or neutrophil collagenase degrading primarily type I collagen and to lesser extent also collagens II and III as well as aggrecan [1]. MMP-8 (collagenase-2) functions in connective tissue turnover in various inflammatory processes [1]. MMPs are important molecules for the immune cell recruitment, and they can modify cellular communication and immune responses by proteolytic processing of non-matrix bioactive substrates such as cytokines, chemokines, serpins, apoptosis factors, cell adhesion factors, and complement components [2]. MMP-8 is expressed in neutrophils [3], macrophages [4] and plasma cells [5] but also in a wide variety other cells such as epithelial cells [1] and fibroblasts [3].

In human malignancies, MMP-8 is up-regulated in head and neck squamous cell carcinomas [6], in lung cancer [7], in ovarian carcinomas [8], in breast cancer cell lines [9], as well as in melanoma [10]. Interestingly, *MMP-8* is often mutated in melanoma together with neuroblastoma RAS viral oncogene homolog (*NRAS)* or v-Raf murine sarcoma viral oncogene homolog B1 *(BRAF*) [10], suggesting that wild-type and mutated *MMP-8* might also be associated with different growth and outcome of melanomas in vivo. Serum markers associated with melanoma progression include lactate dehydrogenase (LDH), S100B, melanoma-inhibiting activity (MIA), tumor-associated antigen 90 immune complex, heparin- and chitin-binding lectin YKL-40 ([11, 12] and references there-in), and MMP-9 [13].

The role of IFN-α as an adjuvant treatment in high-risk melanoma has been extensively studied ([14] and references there-in). Only few studies have found survival benefit for patients receiving IFN-α therapy ([15], [16]). However, a consistent finding in previous studies is prolonged disease-free survival in patients receiving adjuvant IFN-α, independently of IFN-α dose and treatment schedule [17]. Furthermore, meta-analyses confirm that there is a small OS benefit in patients receiving IFN-α as adjuvant treatment [17, 18]. These results suggest that there may be a subpopulation of patients highly sensitive to IFN-α therapy. Previously described predictive factors to suggest beneficial effect of IFN-α therapy are disease stage (N1) and tumor ulceration [19].

A number of biological phenomena are known to be associated with IFN-α therapy, including increase in the number of regulatory T cells [20] and serum ferritin levels [21]. In addition, multiplex analysis of serum cytokines suggests that the levels of various immunosuppressive and tumor angiogenic cytokines are affected and may predict responsiveness to IFN [22]. However, only the serum S100B levels [23, 24] have shown some predictive value when analyzed in randomized clinical adjuvant IFN-α trials.

In the present study, we analyzed the serum samples collected from the patients in the Nordic IFN Trial to further elucidate the prognostic and predictive significance of MMP-8 levels in patients with resected high-risk stage IIB or stage III melanoma.

Patients and methods

Patients

Altogether 855 consecutive patients with histologically verified resected cutaneous melanoma were enrolled into the original Nordic IFN Trial between November 11, 1996, and September 1, 2004 [25]. The original study is registered with ClinicalTrials.gov number NCTO1259934. Patient inclusion criteria in the Nordic IFN trial were as follows: a histologically verified cutaneous melanoma, AJCC stage IIB-III and radical surgery, age at diagnosis ≥18 years, ECOG performance status 0–1, normal bone marrow function, liver chemistry, renal function, normal chest X-ray, abdominal ultrasound, and clinical examination.

All patients had provided written or oral informed consent according to the requirements in each participating

country. Patients were randomized in a 1:1:1 ratio to three different study groups: observation only (group A), adjuvant IFN-α2b, 10 million units (MU) flat dose subcutaneously 5 days per week for 4 weeks (induction) followed by IFN-α2b 10 MU subcutaneously 3 days per week for 12 months (group B), or adjuvant IFN-α2b 10 million units (MU) flat dose subcutaneously 5 days per week for 4 weeks (induction) followed by IFN-α2b 10 MU subcutaneously 3 days per week for 24 months (group C). Patients were followed-up for 10 years.

The subcohort of the current study comprised 460 patients, whose serum samples were available for translational analysis. Unfortunately the studied patient cohort is small, because when the Nordic IFN study was started the collection of serum samples was incomplete. Several patients were treated in centers, where collection of translational serum samples was not possible. These 460 patients include patients in Group A with observation only ($n = 147$), those in Group B with intermediate-dose adjuvant IFN-α for 12 months ($n = 155$), as well as Group C who received adjuvant IFN-α for 24 months ($n = 158$). In most analysis of the present study, patients in Group B and C ($n = 313$) were clumped together and compared with those in Group A ($n = 147$).

Measurement of serum MMP-8 levels by immunofluorometric assay (IFMA)

MMP-8 concentrations were analyzed by a time-resolved immunofluorescence assay, as described previously [3]. The monoclonal MMP-8 specific antibodies 8708 and 8706 (Medix Biochemica, Kauniainen, Finland) were used as a catching and tracer antibody, respectively. Europium-chelate was used to label the tracer antibody. The samples were diluted in assay buffer, incubated for 1 h, and followed by incubation for 1 h with the tracer antibody. Enhancement solution was added, and the fluorescence was measured after 5 min using 1234 Delfia Research Fluoremeter (Wallac, Turku, Finland). MMP-8 levels are expressed as ng/ml. The interassay coefficient of variation was 7.3 % ($n = 28$) and detection limit for the assay 0.08 ng/ml [3]. Serum samples from all patients were gathered after surgery and randomization into the study, but before any IFN-treatment or observation. Each serum sample was stored at -80 C until analyzed for MMP-8 content. The mean value of three parallel measurements in a single sample was used as the MMP-8 value in all analysis.

Statistical analysis

Statistical analyses were done using two statistical software packages: IBM SPSS 20.0.1 for Windows (IBM, NY, USA) and STATA/SE 12.1 software (STATA Corp., Texas, USA). Frequency tables were analyzed using the Chi-square test, with likelihood ratio or Fischer's exact test being used to assess the significance of the correlation between the categorical variables. Odds Ratios and their 95 % Confidence Intervals (95 % CI) were calculated where appropriate, using the exact method. Differences in the means of continuous variables were analyzed using nonparametric tests (Mann–Whitney or Kruskal–Wallis) for 2- and multiple independent samples, respectively. Analysis of variance (ANOVA) was only used for deriving the mean values (and their 95 % CI) of each individual stratum. Univariate survival analysis for the outcome measure (DSS, RFS) was based on Kaplan–Meier method, with log-rank (Mantel-Cox) comparison test. To assess the value of serum MMP-8 level (below/above median) as an independent predictor, multivariate survival analysis was performed, using the Cox proportional hazards regression model controlling for the confounding by the following variables: age (continuous), gender (male = 0, female = 1), Breslow thickness (in mm), ulceration (yes = 1, no = 2), stage (II vs. III), and treatment arm (observation vs. adjuvant IFN-α for 1–2 years). In all tests, the values $P < 0.05$ were regarded statistically significant.

We also separately calculated multivariate survival analysis for stage III patients only. These patients were divided into categories 1, 2, or ≥3 positive lymph nodes.

Preliminary results of this manuscript have been orally presented and published as a congress abstract at the Nordic Melanoma Meeting in Malmö, Sweden, 2012.

Results

Patient characteristics

Serum samples for MMP-8 analysis were available from 460 patients, comprising the material of the present study. The median follow-up time for patients alive at the end of the study period was 68.7 months (range 1.64–137.9 months). The key patient characteristics are presented in Table 1.

RFS and OS in different treatment arms

We wanted to confirm that the current patient cohort with serum samples available was similar to the one included in the original Nordic IFN trial. RFS was 21.8 months in observation only Group A ($n = 147$), and 42.0 months in combined adjuvant IFN-α for 12 or 24 months Group B + C ($n = 313$) ($P = 0.063$). OS was 57.8 months in Group A, and 70.6 months in combined Group B + C ($P = 0.289$).

Table 1 Characteristics of the 460 operated high-risk melanoma patients before randomization

Variable	(Total $n = 460$)	Observation ($n = 147$)	Adjuvant IFN-α ($n = 313$)
Age years	48.8 (17–77)	50 (17–76)	52 (22–77)
Men	296 (64 %)	93 (63 %)	203 (65 %)
Women	164 (36 %)	54 (37 %)	110 (35 %)
Performance status			
0	410 (89 %)	123 (84 %)	287 (92 %)
1	48 (10 %)	23 (16 %)	25 (8 %)
Unknown	2 (<1 %)	1 (<1 %)	1 (<1 %)
Breslow mean tumor thickness mm	3.75 (0.16–45)	3.45 (0.5–20)	3.89 (0.16–45)
Ulceration			
Absent	243 (53 %)	81 (55 %)	162 (52 %)
Present	129 (28 %)	36 (25 %)	93 (29 %)
Unknown	88 (19 %)	30 (20 %)	58 (19 %)
AJCC stage			
IIB–IIC	92 (20 %)	30 (20 %)	62 (20 %)
IIIN1	205 (45 %)	67 (46 %)	138 (44 %)
IIIN2	102 (22 %)	31 (21 %)	71 (23 %)
IIIN3	61 (13 %)	19 (13 %)	42 (13 %)

Data are median numbers (range). Tumor ulceration data were missing from 88 (19 %) of patients because recording of tumor ulceration was adopted into routine practice only since 2003

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Serum MMP-8 levels

The median pre-treatment serum level of MMP-8 in the whole cohort of 460 patients was 21.8 ng/ml (range 0.28–809). Using this median MMP-8 level as cutoff, we divided the patient cohort into two groups: (1) those with serum MMP-8 levels ($n = 232$) below the median, hereafter referred to as the "low MMP-8 group" having median serum MMP-8 levels of 11.7 ng/ml (range 0.3–21.8) and (2) patients with MMP-8 levels above the median, referred to as the "high MMP-8 group" ($n = 228$), whose median serum MMP-8 level was 39.7 ng/ml (range 22.4–809). Patient characteristics and serum MMP-8 levels are shown in Table 2.

Serum MMP-8 levels and RFS

Median RFS in the whole patient group ($n = 460$) was 36.8 months. Patients with pre-treatment MMP-8 levels below the median had a median RFS of 45.1 months as compared to 23.4 months for those with MMP-8 levels above the median, but the difference was not statistically significant ($P = 0.17$). When analyzing the effect of treatment according to pre-treatment MMP-8 levels, patients with high MMP-8 had a significant benefit from the adjuvant therapy in contrast to patients with low MMP-8 levels. Median RFS for patients with high MMP-8 and no adjuvant therapy ($n = 73$) was 10.6 months compared to patients that received adjuvant therapy ($n = 155$), in whom median RFS was 36.8 months ($P = 0.027$) (Fig. 1). In the low MMP-8 group, patients without adjuvant treatment ($n = 74$) had a median RFS of 38.2 months as compared with 50.4 months among those receiving adjuvant therapy ($n = 158$) ($P = 0.63$) (Fig. 2).

Patients in the control arm (Group A; $n = 16$) who had both high MMP-8 levels and an ulcerated tumor had a median RFS of 7.6 months as compared to 19.6 months among patients with high MMP-8 levels and ulcerated tumors in the treatment arm (Groups B + C; $n = 44$) ($P = 0.26$).

In a multivariate model, IFN-α therapy was a significant predictor of favorable RFS (HR 0.74; 95 % CI 0.55–0.99; $P = 0.048$), after adjustment for pre-treatment MMP-8 (HR 1.17; 95 % CI 0.88–1.55; $P = 0.28$), gender (HR 1.16; 95 % CI 0.86–1.56; $P = 0.32$), age (HR 1.00; 95 % CI 1.00–1.02; $P = 0.12$), ulceration (HR 1.09; 95 % CI 0.81–1.46; $P = 0.58$), and number of lymph node metastases (0 or ≥1; HR 1.36; 95 % CI 1.17–1.58; $P < 0.0001$).

We also separately analyzed stage III patients ($n = 338$) in a multivariate model, because number of positive lymph nodes is an important prognostic factor for RFS and OS. In this analysis, IFN-α therapy was still a significant predictor of favorable RFS (HR 0.74; 95 % CI 0.55–1.00; $P = 0.05$), after adjustment for pre-treatment MMP-8 (HR 1.20; 95 % CI 0.91–1.59; $P = 0.20$), gender (HR 1.14; 95 % CI 0.85–1.54; $P = 0.39$), age (HR 1.00; 95 % CI 1.00–1.02; $P = 0.16$), ulceration (HR 1.00; 95 % CI 0.74–1.36; $P = 1.00$), and number of lymph node metastases: one (HR 0.79; 95 % CI 0.54–1.18; $P = 0.25$), two (HR 1.39; 95 % CI 0.90–2.13; $P = 0.16$), and three or more (HR 2.13; 95 % CI 1.35–3.36; $P = 0.001$).

Serum MMP-8 levels and OS

Median OS in the whole subcohort ($n = 460$) was 69.5 months. In the low MMP-8 group, median OS was 71.5 months, whereas in the high MMP-8 group, OS was

Table 2 Patient characteristics as related to pre-treatment serum MMP-8 levels

Patients	Median MMP-8 (range)	Low MMP-8 Group ($n = 232$)[a]	High MMP-8 Group ($n = 228$)[b]
All ($n = 460$)	21.7 ng/ml (0.28–809 ng/ml)		
Men ($n = 296$)	22.8 ng/ml (1.48–809 ng/ml)	147	149
Women ($n = 164$)	21.14 ng/ml (0.28–508 ng/ml)	85	79
Breslow ($n = 435$)		219	216
Clark (n = 457)			
II	17.56 ng/ml (7.45–212 ng/ml)	9	8
III	24.68 ng/ml (4.04–809 ng/ml)	49	63
IV	21.13 ng/ml (1.68–508 ng/ml)	109	98
V	24.34 ng/ml (0.28–216 ng/ml)	19	21
VI	18.51 ng/ml (1.48–450 ng/ml)	46	35
Ulceration			
Absent	21.7 ng/ml (0.3–809 ng/ml)	122	121
Present	20.6 ng/ml (3.7–508 ng/ml)	69	60
Unknown	23.2 ng/ml (1.7–204 ng/ml)	41	47
AJCC stage			
IIB–C	16.4 ng/ml (0.28–285 ng/ml)	56	36
III (N1–3)	23.9 ng/ml (1.5–809 ng/ml)	176	192
IIIN1	24.4 ng/ml (1.5–508 ng/ml)	98	107
IIIN2	23.9 ng/ml (1.7–809 ng/ml)	47	55
IIIN3	21.7 ng/ml (3.3–263 ng/ml)	31	30

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[a] Median serum MMP-8 level 11.7 ng/ml (0.3–21.9 ng/ml)

[b] Median serum MMP-8 level 39.7 ng/ml (22.4–809 ng/ml)

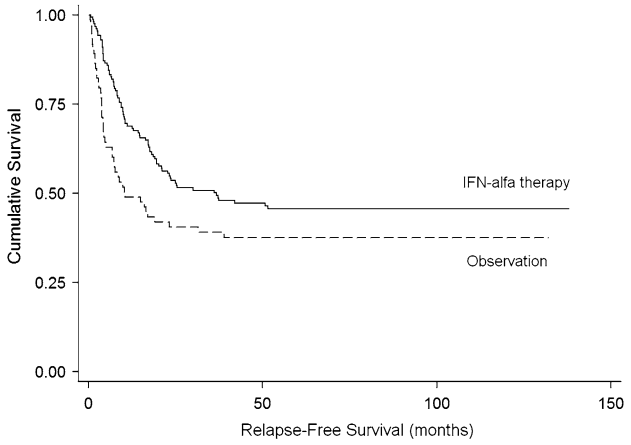

Fig. 1 Relapse-free survival in 228 patients with high serum MMP-8 is associated with treatment arms. *Dash line* = Patients ($n = 73$) in observation only. *Black line* = Patients (155) with adjuvant IFN-α therapy (1 or 2 years); Log-rank $P = 0.027$

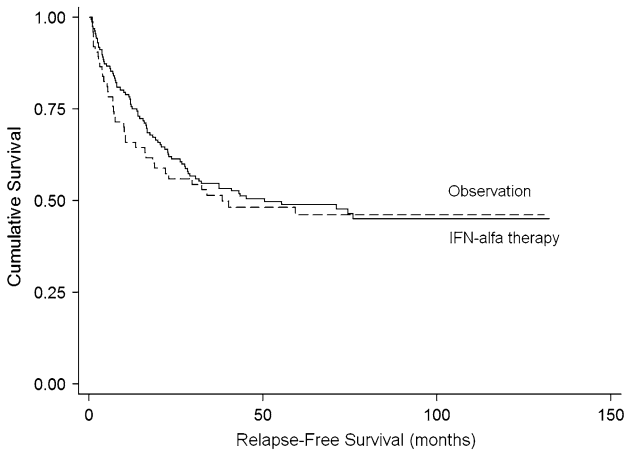

Fig. 2 Relapse-free survival in 232 patients with low serum MMP-8 according to treatment arms. *Dash line* = Patients ($n = 74$) in observation only. *Black line* = Patients (158) with adjuvant IFN-α therapy (1 or 2 years); Log-rank $P = 0.63$

56.6 months ($P = 0.25$). Patients with high MMP-8 levels receiving adjuvant IFN-α therapy ($n = 155$) had a median OS of 71.7 months as compared to 36.7 months among those in the observation group (Group A; $n = 73$) with high MMP-8 levels ($P = 0.13$) (Fig. 3). Patients with low MMP-8 levels in the observation group (Group A; $n = 74$) had a median OS of 94.0 months as compared to 70.6 months among those with low MMP-8 levels receiving adjuvant therapy ($n = 158$) ($P = 0.96$) (Fig. 4).

Duration of adjuvant treatment had a positive impact on OS in the high MMP-8 group. Patients in the observation Group A ($n = 73$) had a median OS of 36.6 months as contrasted to those with 2 years of adjuvant therapy ($n = 76$), who had a median OS of 88.6 months ($P = 0.043$,

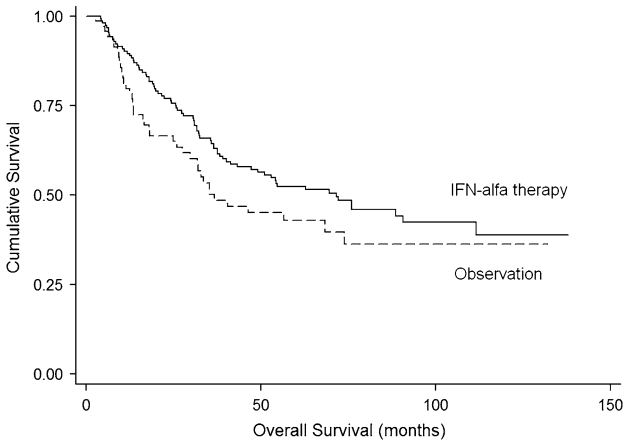

Fig. 3 Overall survival in 228 patients with high MMP-8 according to treatment arms. *Dash line* = Patients ($n = 73$) in observation only. *Black line* = Patients (155) with adjuvant IFN-α therapy (1 or 2 years); Log-rank $P = 0.13$

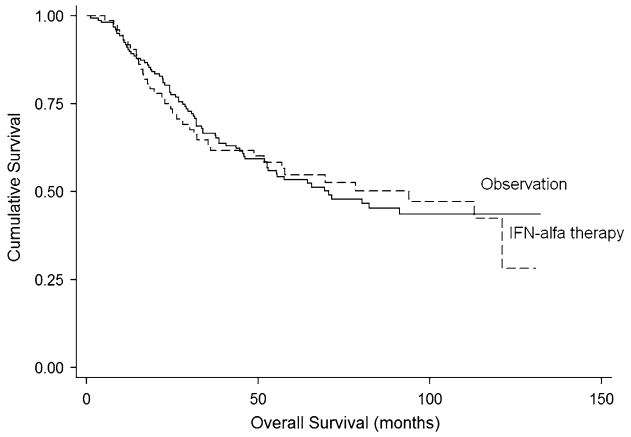

Fig. 4 Overall survival in 232 patients with low MMP-8 according to treatment arms. *Dash line* = Patients ($n = 74$) in observation only. *Black line* = Patients (158) with adjuvant IFN-α therapy (1 or 2 years); Log-rank $P = 0.96$

log-rank). Patients with 1 year of adjuvant therapy ($n = 79$), had a median OS of 47.3 months but the survival difference compared to Group A (observation only) was not significant ($P = 0.50$, log-rank).

In a multivariate model, IFN-α therapy did not reach statistical significance as predictor for OS HR 0.77; 95 % CI 0.56–1.04; $P = 0.09$), following adjustment for MMP-8 (HR 1.14; 95 % CI 0.85–1.52; $P = 0.39$), gender (HR 1.61; 95 % CI 1.17–2.23; $P = 0.004$), age (HR 1.02; 95 % CI 1.00–1.03; $P = 0.004$), ulceration (HR 1.09; 95 % CI 0.81–1.48; $P = 0.56$), and number of lymph node metastases (0 or ≥1; HR 1.41; 95 % CI 1.21–1.64; $P < 0.0001$).

Discussion

Serum LDH is the only recognized melanoma biomarker in the AJCC stage classification [26]. Serum LDH, S100B, and MIA can be used in determination of prognosis of stage IV melanoma [11]. Similarly, increased serum concentrations of angiogenic factors correlate with melanoma progression and survival [23, 24, 27]. In metastatic melanoma, unchanged or declining S100B levels seem to correlate with better treatment response [27].

There is a need for biological markers capable of predicting the outcome of IFN-α adjuvant therapy. Detailed analysis of patients sera or tumor tissue might disclose such predictive markers, as suggested by a recent meta-analyses implicating that IFN-α therapy overall is borderline beneficial in high-risk patients, however, without giving a detailed categorization according to tumor-related characteristics. Yurkovetsky and co-workers [22] performed a multiplexed serum cytokine analysis and described some changes in pro-inflammatory cytokine levels to be associated with RFS. Busse et al. [28] analyzed interferon response genes in peripheral blood of melanoma patients and showed that a decrease in mRNA levels of the interferon stimulated gene 15 (ISG15) was associated with worse outcome during IFN therapy.

MMP-8 is a neutrophil collagenase-2 that has been found to be activated in the chronic inflammation and in the wound healing [1]. High serum levels of MMP-8 seem to be associated with an increase of cardiovascular disease events [29] and increased mortality among patients with interstitial lung disease [30]. In MMP-8-deficient mice, wound healing is delayed [31]. MMP-8 serum and tissue levels are increased in various tumor types [1]. However, MMP-8 is under strict epigenetic control in many cancers, e.g., in glioma and breast cancer cells [32].

We confirmed that RFS and OS in our cohort were similar to those found in the original Nordic IFN study comprising the whole study population. RFS and OS were marginally better in patients treated with IFN-α either 1 or 2 years, in accordance to earlier results [25].

We measured the pre-treatment serum levels of MMP-8 in melanoma patients, who had undergone surgery for high-risk stage IIB-C or stage III disease. All measurements were performed before assignment of study arms (observation only vs. adjuvant IFN-α therapy for 1 and 2 years). In the present study, median serum levels of MMP-8 corresponded well to those described in our previous study on melanoma patients [33] as well as to those in the study on colorectal cancer patients by Väyrynen et al. [34]. The weakness of our study is the limited number of serum samples. We were able to analyze samples of 460 patients, i.e., only 54 % of the original patient population. The sample amount was limited because many patients were treated in

small centers where collection of translational samples was not possible.

Tumor burden and ulceration may be associated with responsiveness to adjuvant IFN-α therapy [19]. Patients with ulcerated tumors and only microscopic lymph node involvement (N1) are known to benefit from adjuvant treatment [19]. The Nordic IFN trial showed that there was a benefit of IFN-α therapy also in patients without ulcerated primary tumors [25]. In the present cohort, patients with ulcerated tumors and high MMP-8 levels had the poorest RFS and OS of all, but this difference did not reach statistical significance.

Adjuvant therapy with intermediate-dose IFN-α was particularly beneficial in patients with high pre-treatment serum MMP-8 levels. Patients with high MMP-8 levels had significantly more favorable RFS if treated with IFN-α as compared to those in the observation group. In contrast, there was no difference in RFS with respect to study arm (observation vs. IFN-α therapy) among patients with low pre-treatment MMP-8 levels.

On the basis of a recent genetic analysis, MMP-8 expression can be either detrimental or beneficial regarding the development and progression of cancer [10, 35]. Interestingly, in 23 % of melanomas, the MMP-8 gene is mutated, implicating that the wild-type MMP-8 has the ability to inhibit melanoma progression [10]. It is not known, whether a mutated MMP-8 gene leads to increase of MMP-8 levels in sera. Furthermore, we do not know whether serum MMP-8 is in an activated or in a latent form. These findings suggest that there may also be other mutated growth-regulatory genes in melanoma, in addition to *BRAF*, *NRAS* and Kirsten rat sarcoma viral oncogene homolog (*KRAS)*.

Taken together, the present results suggest that MMP-8 may act as a serum marker predicting response to adjuvant IFN-α therapy in a subgroup of patients with high-risk malignant melanoma. Patients with high pre-treatment serum MMP-8 levels seem to benefit more from adjuvant IFN-α therapy as compared to patients with low serum MMP-8 levels. However, the observation of IFN-α benefit mainly in the MMP-8 high patient group is hypothesis generating and should be further investigated.

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Conflict of interest None.

References

1. Van Lint P, Libert C (2006) Matrix metalloproteinase-8: cleavage can be decisive. Cytokine Growth Factor Rev 17(4):217–223
2. Gueders MM, Foidart JM, Noel A, Cataldo DD (2006) Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 533(1–3):133–144
3. Hanemaaijer R, Sorsa T, Konttinen YT et al (1997) Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. J Biol Chem 272(50):31504–31509
4. Herman MP, Sukhova GK, Libby P et al (2001) Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. Circulation 104(16):1899–1904
5. Wahlgren J, Maisi P, Sorsa T et al (2001) Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. J Pathol 194(2):217–224
6. Moilanen M, Pirilä E, Grenman R et al (2002) Expression and regulation of collagenase-2 (MMP-8) in head and neck squamous cell carcinomas. J Pathol 197(1):72–81
7. Shah SA, Spinale FG, Ikonomidis JS et al (2010) Differential matrix metalloproteinase levels in adenocarcinoma and squamous cell carcinoma of the lung. J Thorac Cardiovasc Surg 139(4):984–990
8. Stadlmann S, Pollheimer J, Moser PL et al (2003) Cytokine-regulated expression of collagenase-2 (MMP-8) is involved in the progression of ovarian cancer. Eur J Cancer 39(17):2499–2505
9. Köhrmann A, Kammerer U, Kapp M et al (2009) Expression of matrix metalloproteinases (MMPs) in primary human breast cancer and breast cancer cell lines: new findings and review of the literature. BMC Cancer 9:188. doi:10.1186/1471-2407-9-188
10. Palavalli LH, Prickett TD, Wunderlich JR et al (2009) Analysis of the matrix metalloproteinase family reveals that MMP-8 is often mutated in melanoma. Nat Genet 41(5):518–520
11. Gogas H, Eggermont AM, Hauschild A et al (2009) Biomarkers in melanoma. Ann Oncol 20(Suppl 6):vi8–vi13
12. Díaz-Lagares A, Alegre E, Arroyo A et al (2011) Evaluation of multiple serum markers in advanced melanoma. Tumour Biol 32(6):1155–1161
13. Nikkola J, Vihinen P, Vuoristo M-S et al (2005) High serum levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 are associated with rapid progression in patients with metastatic melanoma. Clin Cancer Res 11(14):5158–5166
14. Tarhini AA, Gogas H, Kirkwood JM (2012) IFN-α in the treatment of melanoma. J Immunol 189(8):3789–3793
15. Kirkwood JM, Strawderman MH, Ernstoff MS et al (1996) Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. J Clin Oncol 14(1):7–17
16. Garbe C, Radny P, Linse R et al (2008) Adjuvant low-dose interferon alpha 2a with or without dacarbazine compared with surgery alone: a prospective-randomized phase III DeCOG troal in melanoma patients with regional lymph node metastasis. Ann Oncol 19(6):1195–1201
17. Mocellin S, Pasquali S, Rossi CR, Nitti D (2010) Interferon alpha adjuvant therapy in patients with high-risk melanoma: a systematic review and meta-analysis. J Natl Cancer Inst 102(7):493–501
18. Mocellin S, Lens MB, Pasquali S et al (2013) Interferon alpha for the adjuvant treatment of cutaneous melanoma. Cochrane Database Syst Rev 6:CD008955

19. Eggermont AM, Suciu S, Testori A et al (2012) Long-term results of the randomized phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. J Clin Oncol 30(31):3810–3818
20. Ascierto PA, Napolitano M, Celentano E et al (2010) Regulatory T cell frequency in patients with melanoma with different disease stage and course, and modulating effects of high-dose interferon-alpha 2b treatment. J Transl Med 8:76
21. Bouwhuis MG, Collette S, Suciu S et al (2011) Changes of ferritin and CRP levels in melanoma patients treated with adjuvant interferon-α (EORTC 18952) and prognostic value on treatment outcome. Melanoma Res 21(4):344–351
22. Yurkovetsky ZR, Kirkwood JM, Edington HD et al (2007) Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha2b. Clin Cancer Res 13(8):2422–2428
23. Tarhini AA, Stuckert J, Lee S et al (2009) Prognostic significance of serum S100B protein in high-risk surgically resected melanoma patients participating in Intergroup Trial ECOG 1694. J Clin Oncol 27(1):38–44
24. Bouwhuis MG, Suciu S, Kruit W et al (2011) Prognostic value of serial blood S100B determinations in stage IIB-III melanoma patients: a corollary study to EORTC trial 18952. Eur J Cancer 47(3):361–368
25. Hansson J, Aamdal S, Bastholt L et al (2011) Two different durations of adjuvant therapy with intermediate-dose interferon alfa-2b in patients with high-risk melanoma (Nordic IFN trial): a randomized phase 3 trial. Lancet Oncol 12(2):144–152
26. Balch CM, Gershenwald JE, Soong SJ et al (2009) Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 27(36):6199–6206
27. Utikal J, Schadendorf D, Ugurel S (2007) Serologic and immunohistochemical prognostic biomarkers of cutaneous malignancies. Arch Dermatol Res 298(10):469–477
28. Busse A, Rapion J, Fusi A et al (2013) Analysis of surrogate gene expression markers in peripheral blood of melanoma patients to predict treatment outcome of adjuvant pegulated interferon alpha 2b (EORTC 18991 side study). Cancer Immunol Immunother 62(7):1223–1233
29. Tuomainen AM, Nyyssönen K, Laukkanen JA et al (2007) Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. Arterioscler Tromb Vasc Biol 27(12):2722–2728
30. Oka S, Furukawa H, Shimada K (2013) Serum biomarker analysis of collagen disease patients with acute-onset diffuse interstitial collagen disease. BMC Immunol 14:9
31. Gutiérrez-Fernández A, Inada M, Balbín M et al (2007) Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8). FASEB J 21(10):2580–2591
32. Chernov AV, Baranovskaya S, Golobkov VS et al (2010) Microarray-based transcriptional and epigenetic profiling of matrix metalloproteinases, collagens, and related genes in cancer. J Biol Chem 285(25):19647–19659
33. Vihinen P, Koskivuo I, Syrjänen K et al (2008) Serum matrix metalloproteinase-8 is associated with ulceration and vascular invasion of malignant melanoma. Melanoma Res 18(4):268–273
34. Väyrynen JP, Vornanen J, Tervahartiala T et al (2012) Serum MMP-8 levels increase in colorectal cancer and correlate with disease course and inflammatory properties of primary tumors. Int J Cancer 131(4):E463–E474
35. Gutiérrez-Fernández A, Fueyo A, Folgueras AR et al (2008) Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumour cell adhesion and invasion. Cancer Res 68(8):2755–2763