Dear Sir/Madam:
Enclosed are the galley proofs of your article for ANTICANCER RESEARCH.

We would like to call your attention to the following:
1. Please read thoroughly, correct, and return the proofs to the Editorial Office within 24 hours.
2. Proofs should be returned by the fastest mail service available. Delays in the return of these proofs will necessitate the publication of your paper in a later issue of the journal. You may also send the proofs by fax or e-mail.
3. Please read the entire manuscript carefully to verify that no changes in meaning have been introduced into the text through language improvements or editorial corrections.
4. Corrections should be limited to typographical errors.
5. To promote rapid publication of articles in ANTICANCER RESEARCH, figures are not sent to the Author(s) unless alterations have been made through the editing process.
6. Should you require reprints, PDF files, issues or subscriptions, please fill the attached reprint order form.
7. Should you require information about your article (publication date, volume, page numbers, etc) please call: +30-22950-52945 or send an e-mail to journals@iiar-anticancer.org.
8. Please provide your complete address (not P.O.B.), telephone and fax numbers.
9. Please feel free to contact us with any queries that you may have (Tel./Fax: +30-22950-53389 or +30-22950-52945, e-mail: journals@iiar-anticancer.org).

Thank you for taking the time to study these guidelines.
Yours,

J.G. Delinassios
Managing Editor

Enclosures
Increased Expression of Macrophage Migration Inhibitory Factor During Progression to Hypopharyngeal Squamous Cell Carcinoma

STÉPHANIE CLUDTS1, CHRISTINE DECAESTECKER2, BRYON JOHNSON3, JÉRÔME LECHIEN1, XAVIER LEROY4, NADÈGE KINDT1, HERBERT KALTNER5, SABINE ANDRÈ5, HANS-JOACHIM GABIUS5 and SVEN SAUSSEZ1,6

1Laboratory of Anatomy and cellular biology, Faculty of Medicine and Pharmacy, University of Mons-Hainaut, Mons, Belgium; 2Laboratory of Toxicology, Institute of Pharmacy and 6Department of Oto-Rhino-Laryngology, Faculty of Medicine, Free University of Brussels (ULB), Brussels, Belgium; 3Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, U.S.A.; 4Department of Pathology, Faculty of Medicine, Hôpital Claude Huriez and Centre de Biologie-Pathologie-CHRU, Lille, France; 5Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany

Abstract. Background/Aim: To examine the presence of macrophage migration inhibitory factor (MIF) quantitatively in relation to neoplastic progression of hypopharyngeal squamous cell carcinomas (HSCCs). Materials and Methods: The presence of MIF was analysed by quantitative immunohistochemistry in sections of 81 HSCCs, and compared to 15 specimens of tumour-free epithelia (TF_E), 29 low-grade dysplasias (Low_D) and 25 high-grade dysplasias (High_D). In parallel, MIF expression was studied using Western blotting on a series of 19 fresh biopsies. Results: A significant increase in MIF staining intensity (Mean Optical Density) was observed in carcinoma samples compared to TF_E (p<10^-6), Low_D (p=0.0006) or High_D (p=0.0006). Immunohistochemical MIF positivity was significantly higher in HSCCs than in TF_E (p=0.00001) or Low_D (p=0.001). The percentage of MIF-immunopositive cells (labelling index) significantly decreased in parallel with an apparent loss of histological differentiation (p=0.003). Conclusions: This study identified the presence of MIF as a parameter that positively correlates with neoplastic progression of HSCCs and cell differentiation status.

Correspondence to: S. Saussez, M.D., Ph.D., Laboratory of Anatomy, Faculty of Medicine and Pharmacy, University of Mons (Umons), Pentagone 1B-Avenue du Champ de Mars, 6 B-7000 Mons, Belgium. Tel: +32 65373556, Fax: +32 65373557, e-mail: sven.saussez@umons.ac.be

Key Words: Dysplasia, epithelium, hypopharynx, malignancy, squamous cell carcinoma.

The term ‘macrophage migration inhibitory factor (MIF)’ stems from the detection of activity guinea pig T lymphocytes that inhibit random migration of macrophages (reviewed in (1)). The ensuing identification of a wide spectrum of MIF bioactivities in immunity and neuro-endocrinology has fuelled the interest to explore its role in tumourigenesis and malignancy (2, 3). Indeed, this multifunctional protein also appears to be active in this respect, relating to angiogenesis, growth control and motility (4-6). Of special note, MIF counteracts apoptosis induction by p53 and redox stress, and promotes tumour cell survival via the PI3K/Akt pathway (6-8). Fittingly, the impact of microRNA-451 on reducing proliferation of gastrointestinal cancer cells and enhancing their radiosensitivity can be explained by downregulating MIF (9). Its presence and secretion from various tumour types, such as breast and colon carcinoma, melanoma, adenocarcinoma and haematoma of the lung as well as malignancies of the central nervous system, has prognostic implications, providing a solid clinical platform to systematically test the relevance of MIF presence as inferred by in vitro studies (6, 10-18).

For squamous cell carcinoma the status of knowledge regarding MIF is currently still rather limited. Oesophageal cancer cell lines reacted to MIF exposure with a dose-dependent increase in VEGF and IL-8 secretion, and analysis of tumour specimens revealed upregulated expression in cancer and a correlation between numbers of MIF+ cells, levels of apoptosis/differentiation and lymph node status (19). It is an entirely open question as to what extent MIF is present in hypopharyngeal cancer and how its expression...
changes during the course of tumour progression. In this respect, MIF monitoring deserves special attention, because the analysis of hyperplasia in colon and lung tumourigenesis has provided initial evidence for an *in vivo* association of MIF with tumourigenesis. Specifically, upregulation of MIF is a characteristic of atypical adenomatous hyperplasia of colon and lung adenomas, and abrogation of MIF expression in the ApcMin mouse model resulted in reduced adenoma incidence and size (20-22).

Using a polyclonal anti-MIF antibody preparation, this study applied quantitative immunohistochemistry to a series of 81 cases of stage IV hypopharyngeal squamous cell carcinoma (HSCC). For comparison, normal controls (15 cases of tumour-free epithelia, TF_E) and dysplastic tissue (29 cases of low-grade dysplasias, Low_D, and 25 cases of high-grade dysplasias, High_D) from peritumoural regions were processed to address whether: (i) MIF can be detected in different specimens and (ii) MIF expression is quantitatively altered during disease progression.

### Materials and Methods

**Patient characteristics.** A total of 81 patients with HSCC, who underwent surgery aimed at curative tumour resection, were studied. The patient files were compiled retrospectively (January 1989 to December 2001) from records of the ENT Department at the Hôpital Claude Huriez (Lille, France). Twenty percent of stage IV hypopharyngeal patients presented with positive margins. Description of tumour status was based on the histopathological grade of tumour differentiation (criteria defined in (23)) and the TNM staging classification (24). Detailed information on patient age, gender, tumour histopathology, type of hypopharyngeal surgery, response to treatment at the primary tumour site, follow-up data up to the last patient contact, and disease status were available for 81 patients (Table I).

![Figure 1. Western blotting of tissue extracts from normal pharynx (A-B) and hypopharyngeal carcinomas (C-F) enabled the detection of MIF protein (at the right molecular weight i.e. 12kDa). Variability in the MIF expression may be observed.](image)

<table>
<thead>
<tr>
<th>Mean age: 55 years (range between 40 and 78 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Localisation:</strong></td>
</tr>
<tr>
<td>Periصن sinus</td>
</tr>
<tr>
<td>Postcricoid area</td>
</tr>
<tr>
<td>Posterior wall</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
</tr>
<tr>
<td>Well-differentiated</td>
</tr>
<tr>
<td>Moderately differentiated</td>
</tr>
<tr>
<td>Poorly differentiated</td>
</tr>
<tr>
<td><strong>TNM stage</strong></td>
</tr>
<tr>
<td>T2N2</td>
</tr>
<tr>
<td>T3N2</td>
</tr>
<tr>
<td>T4N0</td>
</tr>
<tr>
<td>T4N1</td>
</tr>
<tr>
<td>T4N2</td>
</tr>
<tr>
<td>T4N3</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Partial pharyngolaryngectomy</td>
</tr>
<tr>
<td>Total pharyngolaryngectomy</td>
</tr>
<tr>
<td>Circular pharyngolaryngectomy</td>
</tr>
<tr>
<td>Esophagopharyngolaryngectomy</td>
</tr>
<tr>
<td>Patients presented larynx cartilage invasion</td>
</tr>
<tr>
<td>Neck dissections in 81 patients</td>
</tr>
<tr>
<td>Patients with extranodal spread</td>
</tr>
<tr>
<td><strong>Recurrent</strong></td>
</tr>
<tr>
<td>Local recurrence: 17 cases</td>
</tr>
<tr>
<td>Distant recurrence: 11 cases</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
</tr>
<tr>
<td>Patients with clinical follow-up data</td>
</tr>
<tr>
<td>Second primaries</td>
</tr>
<tr>
<td>lung cancer</td>
</tr>
<tr>
<td>prostate cancer</td>
</tr>
<tr>
<td>kidney cancer</td>
</tr>
<tr>
<td>Head and neck second primaries</td>
</tr>
<tr>
<td><strong>Deaths</strong></td>
</tr>
<tr>
<td>Caused by the HNSCC</td>
</tr>
<tr>
<td>Without relation with HNSCC</td>
</tr>
<tr>
<td>caused by second primary</td>
</tr>
<tr>
<td>caused by medical disease</td>
</tr>
<tr>
<td>caused by unknown origin</td>
</tr>
</tbody>
</table>

All tissue specimens were from patients who did not undergo chemotherapy and/or radiotherapy prior to surgery. All stage IV hypopharyngeal SCC patients received additional post-operative radiotherapy. The HSCC patient cohorts were thus nearly homogeneous in terms of histopathological and clinical criteria. Patients suffering from SCCs localised at other sites of the head and neck area were excluded from the study. This study was approved by the local Institutional Review Board.

**Antibody preparation and quality controls.** Human MIF was purified by affinity chromatography as described previously (25), followed by high-resolution preparative gel electrophoresis. The protein was rigorously checked for purity by two-dimensional gel electrophoresis and mass spectrometry, and the purified MIF was used as an antigen for raising polyclonal antibodies. Antibody titres in rabbit serum were regularly monitored using ELISA. Serum was fractionated for immunoglobulin G by chromatography on an Agarose Fast Flow resin bound with recombinant protein A (Upstate Biotechnology, Millipore, Schwalbach, Germany).
Western blotting. Tissue from hypopharyngeal TF_E and HSCC biopsies was homogenised in 2.5 vol Tris-sucrose buffer (25 mM Tris-HCl, pH 7.4, containing 250 mM sucrose) with 5 mM EDTA and protease inhibitors. Tissue homogenates were centrifuged at 700xg for 10 min, then NaCl and MgSO_4 were added to the supernatants to reach final concentrations of 100 mM and 1 mM, respectively. These supernatants were spun at 100,000xg for one hour. Homogenisation and centrifugation were carried out at 4°C. Supernatants from the ultracentrifugation step were used as cytosolic fractions and assayed for protein content. For Western blot analysis, 20 μg of cytosolic proteins resolved by SDS-PAGE on 8% T acrylamide-bisacrylamide gels. After separation, proteins were electrotransferred from the gels onto nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany). Non-specific protein-binding sites on the membranes were blocked for three hours at room temperature using a blocking buffer (blotto A) [TBS buffer (10 mM Tris-HCl, pH 8, 150 mM NaCl) containing 5% non-fat milk and 0.05% Tween-20]. Membranes were then incubated overnight at 4°C with the anti-MIF antibody (2 μg/ml). Exposure to the anti-MIF antibody was followed by incubation for 2 hours at room temperature with a peroxidase-conjugated goat anti-rabbit secondary. Finally, after 15 seconds of incubation in the presence of BM chemiluminescence blotting substrate (POD), immunoreactive bands were visualised following exposure of the membrane to a sensitive film (Amersham Hyperfilm ECL, GE Healthcare Limited, Buckinghamshire, UK). Biotinylated molecular weight markers were run and blotted in parallel for internal calibration.

Immunohistochemistry. All tumour samples were fixed for 24 h in 10% buffered formaldehyde, dehydrated and embedded in paraffin. Immunohistochemistry was performed on 5 μm-thick sections mounted on silane-coated glass slides, as previously detailed (26). Before starting the immunohistochemistry protocol, dewaxed tissue sections were briefly subjected to microwave pretreatment in a 0.01 M citrate buffer (pH 6.0) for 2x5 min at 900 W. The sections were then incubated with a solution of 0.4 % hydrogen peroxide for 5 min to block endogenous peroxidase activity, rinsed in phosphate-buffered saline (PBS; 0.04 M Na_2HPO_4, 0.01 M KH_2PO_4 and 0.12 M NaCl, pH 7.4) and successively exposed for 20 min to solutions containing avidin (0.1 mg/ml in PBS) and biotin (0.1 mg/ml in PBS) to avoid false-positive staining reactions resulting from endogenous biotin. After thorough washing with PBS, the sections were incubated for 20 min with a solution of 0.5 % casein in PBS and sequentially exposed at room temperature to solutions of: (i) the...
specific primary anti-MIF antibody, (ii) the corresponding biotinylated secondary antibody (polyclonal goat anti-rabbit IgG) and (iii) the avidin-biotin-peroxidase complex (ABC kit). Between incubation steps, the samples were thoroughly washed to remove unbound proteins. Presence of antigen (MIF) in the sections was visualised by incubation with the chromogenic substrates containing diaminobenzidine and H₂O₂. After rinsing, the sections were counterstained with luxol fast blue and mounted. To exclude antigen-independent staining, the incubation step with primary antibody was omitted from the controls. In all cases these controls were negative. The biotinylated secondary antibodies and ABC kit were obtained from DakoCytomation (Glostrup, Denmark).

Definition of low- and high-grade epithelial dysplasias. Morphological characteristics of dysplasia include increased cellular density associated with a large number of mitotic figures in the vicinity of the basal layer, irregular maturation, loss of polarity and dyskeratosis. Cytologic dysplasia is characterised by an increased ratio of nuclear to cytoplasmic area, anisocytosis, poikilocytosis, nuclear polymorphism, chromatin condensation and large nucleoli; features sometimes associated with atypical mitotic figures. Low-grade dysplasia, consisting of mild and moderate dysplasia, presents atypical features extending over the lower or middle third of the epithelium. High-grade dysplasia and carcinoma in situ extend over the entire thickness of the epithelium (27).

Computer-assisted microscopy. Following the immunohistochemical steps, quantitative features of MIF staining were determined using a computer-assisted KS 400 imaging system (Carl Zeiss Vision, Hallbergmoos, Germany) connected to a Zeiss Axioplan microscope, as detailed previously (26). For each microscopic field, the analysis was focused on neoplastic cells or tumour-free epithelia using computer-assisted morphometry after interactive identification. These tissue areas were delimited precisely with the computer mouse. In each case, 15 fields were scanned covering a surface area ranging from 60,000 to 120,000 μm². The quantitative analysis of immunohistochemical staining yielded data on the following two variables: (i) the labelling index (LI), defined as the percentage of positive cells, and (ii) the mean optical density (MOD), defined as the staining intensity of positive cells (26). For each type of dysplasia (low or high grade), the respective fields within the peritumoural areas were defined by one of the authors (XL) with expertise in this diagnostic procedure.

Data analysis. Independent groups of quantitative data were compared using the non-parametric Kruskall-Wallis (more than two groups) or Mann-Whitney U tests (two groups). In the case of more than two groups, post-hoc tests (Dunn procedure) were used to compare pairs of groups (to avoid multiple comparison effects).
Relationships between the qualitative (or ordinal) variables analysed were studied by contingency tables. The level of significance of correlations was evaluated by the $\chi^2$ or the exact Fisher test (in the 2×2 cases). A decision-tree approach was systematically applied to disclose threshold values aiming at separation of patient groups with and without recurrence (28). Statistical analyses were carried out using Statistica software (Statsoft, Tulsa, USA).

Results

Detection of MIF by Western blotting. In the first step, tissue extracts were comparatively analysed for the presence of MIF by Western blotting and immunodetection. Normal oropharyngeal epithelium (after resection for snoring surgery) and tumour-free hypopharyngeal epithelium (after resection of hypopharyngeal cancer) served as normal controls. All surgical specimens were processed immediately after surgery. Following a standard protocol for Western blotting, the characteristic band for MIF was detected (Figure 1), validating the use of the anti-MIF antibody preparation for further work on tissue sections (Figure 1). Staining intensity indicated variability, with a possible trend towards increased intensity in tumour specimens. These results indicated that a more detailed immunohistochemical analysis was warranted.

MIF in normal oro- and hypopharyngeal epithelia. Normal pharyngeal epithelia and tumour-free epithelia served as a reference for detecting any disease-associated changes. Histologically normal mucosa generally stained weakly for MIF in the intermediate and superficial layers (Figure 2A). Immunoreactivity in the basal cellular layer was absent or low.

MIF during tumor progression of HSCCs. In order to detect disease-associated changes for MIF-dependent parameters (LI and MOD), sections of dysplasias and carcinomas were studied under conditions identical to those used in examining normal mucosa. Staining profiles of sections of tumour-free epithelia (15 cases of tumour-free epithelia, TF_E), dysplasias (29 cases of low-grade dysplasias, Low_D and 25 cases of high-grade dysplasias, High_D) and HSCCs (81 cases of stage IV hypopharyngeal carcinomas, CA) revealed a marked increase in MIF expression in High_D and HSCC samples in comparison to Low_D and normal mucosa (Figure 2). The disparity noted from visual inspection of the entire panel of cases was next subjected to quantitative analysis. Staining intensity (MOD) and the extent of tissue positivity (LI) were determined, and the data were analysed by statistical evaluations. Significant changes were observed for both MIF MOD and LI (Kruskall-Wallis: $p<10^{-6}$ for both) (Figure 3A and 3B). Using post-hoc comparisons for pairs of groups, a significant increase in MIF MOD was observed in CA samples compared to TF_E ($p<10^{-6}$), Low_D ($p=0.0006$) or High_D ($p=0.0006$) (Figure 3A). MIF LI, expressed as percentage of cells, was significantly higher in HSCCs than in TF_E ($p=0.00001$) or Low_D ($p=0.001$) (Figure 3B).

Immunohistochemical detection of MIF in stage IV hypopharyngeal SCCs and correlation with clinical features

Visual inspection indicated a correlation between MIF expression levels and the degree of tumour differentiation. An example of the comparison between well- and poorly differentiated patient tissue is shown in Figure 4. The percentage of MIF-immunopositive cells significantly decreased in parallel with an apparent loss of histological differentiation (Figure 4; Mann-Whitney test: $p=0.003$). Further data processing provided no evidence for a significant association with any of the clinical features detailed in Table I (namely tumour location, T status, N status and recurrence status). Monitoring the subcellular sites, cytoplasmic MIF staining was observed in the large majority (89%) of the cases. Some samples showed both cytoplasmic and nuclear MIF localisation.

Discussion

MIF is a potent multifunctional protein that affects diverse types of cells. Its association with tumourigenesis, its increased expression in several different tumour types relative to non-malignant controls, as well as the reduction of tumour cell growth and angiogenesis when MIF activity is impaired, prompted this study to examine the MIF presence in hypopharyngeal cancer using Western blotting and immunohistochemistry. Using MIF polyclonal antibodies, the presence of MIF in tissue extracts and its localisation in tissue samples were determined. Inter-tumour heterogeneity was revealed by both methods. The labelling index positively correlated ($p=0.003$) with the degree of differentiation. Therefore, frequency of immunopositive cells is an indicator of increased cell maturation in hypopharyngeal cancer, whereas a negative correlation was reported previously in oesophageal squamous cell carcinoma (18). It appears that different tumour types can present divergent relationships between MIF and cellular differentiation status. Consequently, the disparity among squamous cell carcinomas documented herein precludes any extrapolations even among histogenetically related tumours.

Quantitative monitoring of MIF expression in tissue sections during the course of disease, to the appearance of malignant tissue, demonstrated a marked upregulation. Starting from normal tissue and proceeding to specimens with two different levels of dysplasia enabled the detection of a significant enhancement of MIF expression preceding malignancy. This pattern of expression is in agreement with a protumoural activity of MIF in vivo, even though the MIF-dependent parameters were not able to serve as a prognostic
indicator. The presented histopathological results provide the impetus for further in vitro testing to delineate the mechanisms of MIF functionality. Taking into account the data of the present study, future experiments could include similar assays with other effectors that show a similar course of expression, as they may functionally cooperate with MIF. The anti-apoptotic protein, galectin-3 is an intriguing candidate. In addition to similar elevated expression profiles in well-differentiated hypopharyngeal carcinomas, the potential functional cooperation between galectin-3 and MIF is supported by a similar correlation in recurrent cholesteatomas (26, 29, 30). Both proteins also share subcellular localisation in the cytoplasm and non-classical secretion (13, 26). By acting through different targets, such as CD74 or bel-2, and different signalling pathways, galectin-3 and MIF may exert additive or synergistic effects (6). This possibility, derived from histopathological analyses, may guide future experimental work, including testing individual proteins or combinations of growth regulators, for example by reducing expression or blocking binding of these proteins to their respective receptors.

In conclusion, this study reported increased MIF expression in hypopharyngeal squamous cell carcinoma with predominantly cytoplasmic localisation. The labelling index positively correlated with the degree of cell differentiation, and expression was upregulated during tumour progression. These characteristics are shared by the adhesion/growth-regulatory protein galectin-3, intimating combined functional assays in vitro to explore the potential for additive/synergistic functionalities.

Acknowledgements

This study was supported in part by an EC Marie Research Training Network grant (contract no. MCRRTN-CT-2005-19561). S. Saussez is the recipient of a grant from the Fondation Vésale (Brussels, Belgium). The expert technical assistance of G. Ninfa is gratefully acknowledged.

References

Dr. S. Saussez

Re: Your manuscript No. 11758-C entitled «Increased Expression of...»

Dear Dr,

Referring to your above manuscript for publication in AR, please allow us to use this form letter in reply:

1. **Referee’s recommendations:**
   - ■ Urgent to be published immediately.
   - □ Accepted in the presented form.
   - □ Accepted with minor changes.
   - ■ Accepted with grammatical or language corrections.
   - □ Remarks:

2. **Excess page charges.**
   - ■ Your article has approx. 7 printed pages and is in excess of the allotted number by approx. 3 printed pages. The charges are EURO € 180 per excess page, totalling EURO € 540. We ask you to confirm acceptance of these charges.
   - ■ Your article includes 2 pages with color figures. The charges are EURO € 650 per color page, totalling EURO € 650 (-50% Discount)
   - ■ Our invoice will be sent by e-mail to the corresponding author.

3. ■ Your article will appear in Volume 30, Issue No. 9, 2010

4. ■ Please order your reprints now. This will facilitate our prompt planning of future issues and rapid publication of your article. Reprints will be delivered by air mail within one month from publication.

We would appreciate your prompt reply.

With many thanks,

Yours sincerely,

J.G. Delinassios
Managing Editor

EDITORIAL OFFICE: INTERNATIONAL INSTITUTE OF ANTICANCER RESEARCH
1st km Kapandritiou - Kalamou Rd., Kapandriti, P.O.B. 22, Attiki 19014, Greece. Tel.: 0030-22950-52945; Tel & Fax:0030-22950-53389; e-mail: journals@iiar-anticancer.org
Please type or print the requested information on the reprint order form and return it to the Editorial Office by fax or e-mail.

Reprints must be paid for in advance.
If your paper is subject to charges for excess pages or color plates, please add these charges to the payment for reprints.
The reprints are not to be sold.

### PRICE LIST FOR REPRINTS WITHOUT COVER

<table>
<thead>
<tr>
<th>Page length</th>
<th>PDF file</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>1000</th>
<th>1500</th>
<th>2000</th>
<th>3000</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4pp</td>
<td>167</td>
<td>335</td>
<td>387</td>
<td>438</td>
<td>503</td>
<td>554</td>
<td>851</td>
<td>1135</td>
<td>1470</td>
<td>2038</td>
<td>3225</td>
</tr>
<tr>
<td>5-8</td>
<td>219</td>
<td>438</td>
<td>503</td>
<td>580</td>
<td>645</td>
<td>722</td>
<td>1083</td>
<td>1445</td>
<td>1832</td>
<td>2554</td>
<td>4012</td>
</tr>
<tr>
<td>9-12</td>
<td>277</td>
<td>554</td>
<td>619</td>
<td>709</td>
<td>787</td>
<td>877</td>
<td>1341</td>
<td>1780</td>
<td>2219</td>
<td>3096</td>
<td>4824</td>
</tr>
<tr>
<td>13-16</td>
<td>354</td>
<td>709</td>
<td>787</td>
<td>890</td>
<td>993</td>
<td>1096</td>
<td>1625</td>
<td>2141</td>
<td>2657</td>
<td>3676</td>
<td>5715</td>
</tr>
<tr>
<td>17-20</td>
<td>419</td>
<td>838</td>
<td>929</td>
<td>1032</td>
<td>1148</td>
<td>1277</td>
<td>1883</td>
<td>2451</td>
<td>3044</td>
<td>4244</td>
<td>6527</td>
</tr>
</tbody>
</table>

For reprints with cover: Please add EURO 140.00 per 100 copies.
Postal: Please add 5% on the above prices.

### Reprint Order Form

Of my paper No. 11758-C comprising 7 printed pages, entitled «Increased Expression of...»
accepted for publication in ANTICANCER RESEARCH Vol. 30 No. 9

☐ I require a total of copies at EURO

☐ I do not require reprints.

☐ Please send me a PDF file of the article at EURO.

☐ Please send me a copy of this issue containing my paper at EURO 45.00.

☐ Please enter my personal subscription to ANTICANCER RESEARCH at the special Author’s price of EURO 417.00 (print or online); EURO 500.00 (print & online) (☐ Year: 2009).

☐ A check for the above amounts payable to J.G. Delinassios, Executive Publisher of Anticancer Research Journal, is enclosed.

☐ Please send an invoice to:

For EC countries: Please give your VAT number:

City and Date: Signature:

Exact postal address:

Tel:

Fax: