Abstract. Background: Human anti-mouse antibody (HAMA)-IgM and IgG in ovarian cancer patients treated with intraperitoneal (i.p.) 90Y-muHMFG1 as consolidating therapy were analyzed for a relationship with outcome of disease. Patients and Methods: Serial serum samples from 208 ovarian cancer patients participating in a phase III trial of i.p. 90Y-muHMFG1 and 25 controls were analyzed for HAMA-IgM and HAMA-IgG. Results were correlated with time to, and location of, disease recurrence. Results: Patients receiving i.p. 90Y-muHMFG1 developed a rapid HAMA-IgM peak (week 4 to 8), followed by a HAMA-IgG peak 2-4 weeks later. HAMA levels in the control group remained unchanged. Early maximum HAMA-IgG peaks were associated with early relapse [hazard ratio (HR), 0.975; 95% confidence interval (CI) 0.956 to 0.995; p=0.012]. Patients with a HAMA-IgG maximum before or at 8 weeks were at significantly higher risk for disease recurrence (HR, 1.6; 95% CI 1.1 to 2.5; p=0.021) as compared to patients with a HAMA-IgG maximum after 8 weeks. Conclusion: Besides time point of maximum HAMA-IgG, no evident relation could be found between HAMA-IgM or HAMA-IgG development and time to relapse or location of recurrence.

Despite advances in the treatment of ovarian cancer, it is still the leading cause of death from gynecological malignancies (1). New strategies to improve overall survival are needed and radioimmunotherapy (RIT) could be one of the options.

Human adenocarcinomas overexpress the transmembrane glycoprotein MUC1, which is normally expressed on the apical surface of glandular epithelial cells (2). Ninety percent of epithelial ovarian cancer expresses high levels of MUC1 (3). MUC1 in cancerous tissue is antigenetically different from the protein in normal tissue as a result of aberrant glycosylation, which leads to the exposure of the peptide core of the mucin with its immunodominant repetitive amino acid sequence (2). The murine IgG1 monoclonal antibody (mAb) HMFG1 recognizes this specific epitope of MUC1 on the apical surface of ovarian cancer cells (4). Several studies have used radiolabeled HMFG1 for imaging and therapeutic targeting of ovarian cancer lesions in the treatment of ovarian cancer (5, 6). Administration of murine mAbs may provoke the development of human anti-mouse antibodies (HAMA) (7). Sero-conversion frequencies of HAMA development in patients with solid tumors range from 50-75% after exposure to murine mAbs (7). Although the consequences of the presence of HAMA are usually minor, subsequent treatment of HAMA-positive patients with mAbs may be affected by rapid clearance of the administered antibody from the blood. Several studies reported an association between the development of antibody-specific HAMA and prolonged survival (8-10). Recently, a large phase III clinical study targeting ovarian cancer with intraperitoneal (i.p.) 90-yttrium labeled murine HMFG1 (90Y-muHMFG1) showed that there was no disease-free or overall survival benefit in patients receiving 90Y-muHMFG1 compared to those receiving standard therapy (11). However, there was a significant difference in the localisation of disease recurrence as patients receiving i.p. 90Y-muHMFG1 had significantly fewer intraperitoneal disease recurrences, and time to this type of recurrence was longer than that of patients in the control group (12).

These observations led us to investigate whether the development of HAMA correlates with localization and time of recurrence in the 90Y-muHMFG1-treated group. For this purpose, we used two recently developed enzyme-linked immunosorbent assays (ELISAs) for specific quantification of the HAMA-IgM and the IgG response in these patients (13).
Patients and Methods

Study design and population. Serial serum samples of ovarian cancer patients in complete clinical remission participating in a phase III trial of i.p. ⁹⁰Y-muHMFG1 (11) were analyzed for the presence of HAMA-IgM and HAMA-IgG directed against the muHMFG1. Of the 224 patients treated with a single i.p. administration of 25 mg ⁹⁰Y-muHMFG1, 208 patients were available for analysis. From the 223 patients who only received standard treatment, we randomly selected 25 patients to serve as controls. The patients were recruited in 74 centers in 17 different countries, in three different continents (Europe, Oceania and Northern America). Patients enrolled in the study after having given written informed consent; procedures were followed in accordance with the Declaration of Helsinki.

Serial serum samples were taken before (week 0, baseline values), at 1, 4, 8, 12 weeks after ⁹⁰Y-muHMFG1 injection, and in three-monthly intervals thereafter until the end of follow-up. Venous blood was collected from an antecubital vein, allowed to clot, centrifuged for 10 minutes at 2000 × g, and serum was stored at −70°C until analyzed. Time to disease recurrence was measured as the number of days between second-look laparoscopy and the date of documented disease recurrence up to the end of the study.

HAMA-IgM and HAMA-IgG ELISAs. Circulating HAMA-IgM and HAMA-IgG antibodies were measured with two ELISAs that were developed in our laboratory as previously described (13). Briefly, coating antibody HMFG1 (Antisoma Research Ltd, London, UK) and bovine serum albumin (BSA) were absorbed onto microtitre plates. Serum samples were tested at dilutions of 1:50, 1:500 and 1:5000, and immunoglobulins bound to HMFG1 were detected with mouse anti-human IgM (Fc)-HRP, or with mouse anti-human IgG-HRP (Southern Biotechnology Associated, Birmingham, AL, USA). Tetramethylbenzidine plus (Kem-En-Tec, Copenhagen, Denmark) was used as substrate, and the reaction was quantified at 450 nm in an ELISA reader (Multiskan Ascent, Lab systems, Helsinki, Finland). Results were expressed as arbitrary units per milliliter (AU/ml).

Statistical methods. Data analysis was performed using SAS 8.0 and SPSS 12.0.1 (SPSS Inc Chicago, IL, USA). Differences in disease outcome were tested with Wilcoxon rank sum test. HAMA-IgM and HAMA-IgG values were analysed in a linear mixed model correlating the individual patients with relapse and location of relapse. To analyse data in a repeated mixed model, the natural logarithm was used to normalize the data. Cox proportional hazards regression model was used to identify prognostic factors (14). P-values of <0.05 were considered significant.

Results

Two hundred and eight ovarian cancer patients who received a single i.p. ⁹⁰Y-muHMFG1 injection and 25 control patients who received standard treatment were evaluated for HAMA-IgM and HAMA-IgG responses. At the start of the present study, all patients were in complete clinical remission after debulking and chemotherapy, as defined at second-look laparoscopy. The median age of the 208 patients in the study group was 54 years (range 21-67 years) and 51 years for the 25 control patients (range 35-79 years). Out of the 208 patients in the study group, 28% (59/208) had FIGO stage I or II and 72% (149/208) had FIGO stage III or IV. In the study group of patients, 48% (100/208) of the patients had no residual disease, 44% (92/208) had residual disease and in 8% (16/208) the residual disease status was unknown. Ninety-eight patients in the study group developed relapse during follow-up with a median time from second-look laparoscopy to relapse of 1 year (range 0.15-4.7 years). Patients in the study group were monitored for HAMA-IgM and HAMA-IgG concentration with a median follow-up of 30 months (range 3 to 72 months).

HAMA-IgM profile. Baseline HAMA-IgM titers in blood, before ⁹⁰Y-muHMFG1 administration had a median value of 997 AU/ml (range 169 to 25,000 AU/ml) in the study group and 1,204 AU/ml (range 207 to 2,758 AU/mL) in the control group. Baseline values did not differ significantly between study and control groups. HAMA-IgM results in the study and the control group during follow-up are displayed in Figure 1A. During follow-up HAMA-IgM in the control group remained unchanged as compared to baseline HAMA-IgM levels. The maximum HAMA-IgM level in the study group (median 31,000 AU/ml; range 1,220 to 5 ×10⁶ AU/m l) was reached at 4 weeks and was 28-fold higher (range 0.6- to 931-fold) than the median baseline values. Ninety-four percent (195/208) of patients in the study group reached a maximum HAMA-IgM concentration within 8 weeks after i.p. ⁹⁰Y-muHMFG1 administration. At the end of the follow-up median HAMA-IgM was 1.4-fold higher (range 1.3- to 1.8-fold) than baseline levels.

HAMA-IgG profile. Baseline HAMA-IgG concentration in blood before ⁹⁰Y-muHMFG1 administration had a median value of 2,317 AU/ml (range 100 to 23,000 AU/ml) in the study group and 2,022 AU/ml (range 577 to 8,063 AU/ml) in the control group. There was no significant difference in baseline HAMA-IgG values between the two groups. Figure 1B shows HAMA-IgG concentrations in the 208 study and 25 control patients during follow-up. Maximum HAMA-IgG values were reached 8 weeks after administration and were 85-fold (median, range 1- to 622-fold) higher than their respective baseline levels. In 92% (192/208) of the study group patients, HAMA-IgG reached its maximum within 24 weeks after i.p. ⁹⁰Y-muHMFG1 administration. By the end of follow-up, the median HAMA-IgG concentration was 12-fold higher (range 3.8- to 41-fold) than the baseline level. During follow-up, HAMA-IgG values in the control group had a median value of 1.754 AU/ml, with a range 339 to 9,809 AU/ml. HAMA-IgG concentrations in the control group were never higher than three times the baseline values.

Correlation of HAMA-IgM and HAMA-IgG with disease-free survival. In order to investigate whether the HAMA-IgM and HAMA-IgG response in the study group is associated with disease recurrence we studied: (i) the time point at which
HAMA-IgM and HAMA-IgG reached their maximum concentrations, (ii) the maximum HAMA-IgM and HAMA-IgG concentrations (peak concentration), and (iii) the individual longitudinal HAMA-IgG and HAMA-IgM measurements during follow-up, and correlated these three parameters with disease outcome. Furthermore, to include time to relapse in our analysis, we compared results of HAMA IgM and IgG between patients with an early (≤1 year, n=43) and a late (>1 year, n=165) relapse.

No association was found between the time point of maximum HAMA-IgM concentration or the peak concentration HAMA-IgM and relapse, time to relapse or location of relapse. Linear mixed model showed no association between the individual HAMA-IgM responses and disease outcome (data not shown). Patients with an early relapse had higher median HAMA-IgM values during week 4 to 12 after 90Y-muHMFG1 administration than did those with a late relapse, but these differences were not statistically significant.

Peak HAMA IgG and the individual HAMA IgG responses in a linear mixed model approach during follow-up were not associated with relapse, time to relapse or location of relapse. Again, median HAMA-IgG values from week 4 to 12 tended to be higher in patients with an early relapse. However, this difference did not reach significance.

Univariate analysis showed a significant association between time point of maximum HAMA-IgG values, as a continuous variable, and time to relapse [hazard ratio (HR) 0.975; 95% confidence interval (CI) 0.956 to 0.995; p=0.012]. To dichotomize this parameter, the group was divided into patients with maximum HAMA-IgG before or at 8 weeks (i.e. the median time) and patients with a maximum after 8 weeks. Patients with a HAMA-IgG peak before or at 8 weeks were at significantly higher risk for disease recurrence (HR 1.7; 95% CI 0.382 to 0.892; p=0.013) as compared to patients with a maximum HAMA-IgG peak after 8 weeks. After correction for residual disease and FIGO stage, patients with a HAMA-IgG maximum before or at 8 weeks were still at significantly higher risk for disease recurrence (HR 1.6; 95% CI 1.1 to 2.5; p=0.021) than those with a HAMA-IgG maximum after 8 weeks. The Kaplan-Meier plot of disease-free survival is shown in Figure 2. When adjusting for stage, in multivariate analysis this difference remained significant (p=0.020). As to be expected, patients with FIGO stage III or higher were at significantly higher risk for recurrence than patients with FIGO stage II or less (HR 2.7; 95% CI 1.5 to 4.6; p=0.001).

Discussion

This study describes the HAMA response in ovarian cancer patients in complete clinical remission after primary treatment who received a single i.p. 90Y-muHMFG1 injection. The majority of patients showed a HAMA-IgM peak within the first
immunogenic as reports on HAMA response after mAb administration vary widely between 40 to 90% of the patients intravenous administration of HMFG1 after which 30-50% of (10, 19, 20). The development of an early HAMA-IgM peak the patients develop HAMA (18). Other mAbs seem less immunogenic as reports on HAMA response after mAb administration vary widely between 40 to 90% of the patients (10, 19, 20). The development of an early HAMA-IgM peak within the first 4-8 weeks after HMFG1 administration and a HAMA-IgG peak approximately 2-4 weeks later is in accordance with the kinetics of an immune response as described in literature after exposure of humans to murine mAb (21). As to be expected, patients in the control group did not show a rise in HAMFG1 HAMA-IgM or HAMA-IgG values, since they did not receive an injection of 90Y-muHMFG1.

The development of antibody-specific HAMA after mAbs exposure has been associated with unexpectedly prolonged survival in cancer patients (8-10, 22). Azinovic et al. (8) demonstrated that higher antibody specific-HAMA titers were associated with longer survival in patients with B-cell malignancies treated with 131I-Lym-1. Recently, a study which treated refractory indolent non-Hodgkin lymphoma patients with 131I-tositumomab (murine mAb) demonstrated an improved survival in the four patients that developed HAMA (23). Miotti et al. (9) showed that 88% of the patients treated with a bispecific antibody OC/TR for ovarian cancer, developed antibody-specific HAMA, but only patients with HAMA titers greater than 150 μg/mL (56% of the HAMA-positive patients) showed an advantage in terms of survival. Earlier studies with i.p. radiolabeled HMFG1 showed a benefit in survival in ovarian cancer patients (6, 15, 16). Unfortunately, the large international multicenter phase III trial of i.p. 90Y-muHMFG1 failed to confirm these findings (11). However, recently we reported that despite the absence of improvement in overall survival observed in the trial, there was a reduced intraperitoneal disease recurrence in patients treated with i.p. 90Y-muHMFG1 (12). Furthermore, time to intraperitoneal disease recurrence in patients in the study group was significantly longer as compared to the control group. In the present study, we were unable to find an association between HAMA-IgM or HAMA-IgG parameters and localization of disease recurrence. The observation of reduced i.p. relapse and delayed i.p. disease recurrence is most likely an effect of the radiation dose given intraperitoneally with 90-Yttrium with which the muHMFG1 was labeled, and not due to a HAMA immune response.

We found an association between the time point at which the HAMA-IgG reaches its maximum and disease recurrence. Patients with an early peak (before or at 8 weeks) were at higher risk for developing relapse as compared to patients with a maximum HAMA-IgG after 8 weeks. These results should be interpreted with caution for there was a wide dispersion in HAMA-IgG values between the patients. Some patients had their peak at week 8 while other patients reached their peak at week 12 with already extremely high HAMA-IgG values at week 8.

In summary, HAMA-IgM and HAMA-IgG development after a single i.p. 90Y-HMFG1 administration occurred in the majority of patients with a primary HAMA-IgM peak within the first 4-8 weeks, followed by a HAMA-IgG peak approximately 2-4 weeks later. In multivariate analysis, patients with a HAMA-IgG maximum before or at 8 weeks were at significantly higher risk for disease recurrence (HR 1.6; 95% CI 1.1 to 2.5; p=0.021) as compared to patients with a HAMA-IgG maximum after 8 weeks.

At present, work is in progress to study the development of anti-idiotypic (Ab2) and anti-anti-idiotypic antibodies (Ab3) in ovarian cancer patients in this study. The production of these anti-idiotypic antibodies has been associated with a beneficial disease outcome in previous studies (24, 25). Further research
into the possible induction of the idiotypic network after injection with murine mAb should be performed to investigate whether the presence of these antibodies improves prognosis in ovarian cancer patients.

References


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