

that receive a dose of $D_{\min}$ or $D_{\max}$. If this is the case, the differential DVH and cumulative DVH should be similar to the DVHs shown in Figure 3, not the left-top DVH plot of Figure 1 shown in Mavroidis and Lind's letter (a similar plot can be found in the Figure 6 in [3]). Here we present Figure 3 to help the reader understand the dose-volume relations used in those examples.

In summary, $\bar{D}$ and EUD are the same concept, which is useful to summarize the biological effect of various complex treatment plans. While outcome modeling with Binomial statistics may better estimate the TCP values at low dose regions for cases with a rather flat dose response ($\gamma \leq 1$), Poisson statistics still provide reliable outcome estimates for radiation therapy in the clinical practice.

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Analyzing Topoisomerase II- α and HER-2/neu co-amplification seems to be of limited value in epithelial ovarian cancer

JOHANNA MÄENPÄÄ¹, MINNA TANNER^{2,3} & JORMA ISOLA³

¹Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Tampere University Hospital, ²Department of Oncology and Radiotherapy, Tampere University Hospital and ³Institute of Medical Technology, University and University Hospital of Tampere, Finland

To the Editor

Recurrent chemoresistant ovarian cancer remains a therapeutic challenge; in randomized Phase III trials the best response rates to chemotherapy have been at

best 12–13% and even so, short-lived [1,2]. Among available agents, pegylated liposomal doxorubicin is a noteworthy alternative [3]. Pegylated liposomal doxorubicin is, however, quite expensive and has

inconvenient side effects as hand-foot syndrome and stomatitis [2]. Consequently, a situation where at least ten patients need to be treated to get one or two responses is definitely less than optimal.

Topoisomerase II α (*TOP2A*) is purported to be the main intracellular target of anthracyclines [4]. In breast cancer, *TOP2A* gene amplification seems to be associated with *HER-2/neu* gene amplification [5]. Indeed, there is evidence that coamplification of these genes in breast cancer renders the cancer cells especially sensitive to anthracyclines [4], although also conflicting results have been published [5]. After Mano et al. showed that evaluating amplification of *TOP2A* and *HER-2/neu* by fluorescent in situ hybridization (FISH) is feasible even in epithelial ovarian carcinoma [6], we decided 1) to perform a preliminary analysis of the rate of *TOP2A* and *HER-2/neu* amplification by chromogenic in situ hybridization (CISH) [7] in epithelial ovarian cancer, and 2) to retrospectively correlate the amplification to the clinical efficacy of pegylated liposomal doxorubicin in patients with recurrent chemoresistant ovarian cancer.

Ten patients with recurrent chemoresistant ovarian cancer gave their informed consent to the study. The median age of the patients was 58.5 years (range 43–79). Of the tumors, seven were serous, two were endometrioid, and one was mucinous. We chose both patients who had responded to pegylated liposomal doxorubicin and patients who had not responded. The study was approved by the local Ethics Committee.

The original paraffin blocks from the primary operation we retrieved from the archives. The digoxigenin-labeled probes for *HER-2/neu* and *TOP2A* needed for CISH were obtained from Zymec Inc. (South San Francisco, CA). The hybridization method used has been described in detail previously [7]. Hybridization was evaluated with an Olympus BX50 microscope (Olympus, Tokyo, Japan) using an X40 objective. Amplification of *HER-2/neu* and *TOP2A* was defined as the presence of six or more copies in more than 30% of nuclei or as presence of an easily identifiable gene copy cluster, the gene copies of which could not be enumerated. The laboratory was blinded as regards to the clinical outcome of the patients.

Four of the carcinomas had responded to pegylated liposomal doxorubicin, as evaluated by the tumor marker Ca-12-5. Three tumors showed stabilization, while the remaining three progressed in spite of the treatment. *HER-2/neu* gene was found to be amplified only in one endometrioid carcinoma

that was unresponsive to doxorubicin. In no instance could we find any amplification of *TOP2A* gene.

Immunohistochemical (IHC) methods were originally used to study the over-expression of *TOP2A* and *HER-2/neu* in cancer cells. Because of difficulties in standardization of IHC, in situ hybridization techniques developed to detect the actual amplification rate have now replaced the IHC methods [8]. Of the in situ hybridization techniques, the newer CISH provides an accurate and practical alternative to the older FISH [4,7].

To our knowledge, Mano et al. were the first to compare *TOP2A* and *HER-2/neu* amplification and expression in epithelial ovarian carcinoma [6]. They found on one hand that the correlation between the expression and the amplification of the respective genes was poor, and on the other hand that there was an excellent correlation between *HER-2/neu* and *TOP2A* amplification. Using more stringent criteria, the amplification rates were 12.5 and 7.8%, respectively. The researches did not compare the amplification rates to in vitro or clinical efficacy of pegylated liposomal doxorubicin.

The article by Mano et al. encouraged us to study the amplification rate of *TOP2A* and *HER-2/neu* in epithelial ovarian cancer and, moreover, to attempt to correlate the rates to the therapeutic effect of pegylated liposomal doxorubicin. Unfortunately, no tumor was found to harbor amplification of *TOP2A*, and even *HER-2/neu* was amplified only once, in a tumor not responsive to doxorubicin. There are obvious shortcomings in our preliminary retrospective study: The samples studied originated from the primary operation, i.e. were chemotherapy-naïve, and pegylated liposomal doxorubicin was started not until the tumors had developed a taxane-platinum resistance. It is possible that the amplification of *TOP2A* and/or *HER-2/neu* is a late phenomenon associated e.g. with the development of a resistance to taxanes and platinum compounds in ovarian cancer. It is, however, difficult to obtain histological samples from recurrent ovarian cancer that is as a rule treated with second-line chemotherapy, rather than re-operated.

In conclusion, the rarity of amplification of *HER-2/neu*, combined with the non-existence of amplification of *TOP2A* and with a poor correlation to the clinical outcome as found in the present study, discourages us from continuing our efforts.

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Motesanib diphosphate (AMG 706), an oral angiogenesis inhibitor, demonstrates clinical efficacy in advanced thymoma

ARUN AZAD¹, REBECCA A. HERBERTSON², DAVID POOK², SHANE WHITE¹,
PAUL L. R. MITCHELL¹* & NIAL C. TEBBUTT^{1,2}*

¹Department of Medical Oncology, Austin Health, Heidelberg, Victoria, Australia and ²Ludwig Institute for Cancer Research, Austin Health, Heidelberg, Victoria, Australia

To the Editor

In May 2000, a 23-year-old male underwent thymectomy and adjuvant radiotherapy for Masaoka stage II thymoma with positive resection margins. In December 2003, CT and positron emission tomography (PET) showed biopsy-proven bilateral pleural recurrence. Left-sided surgical pleurodesis was followed by four cycles of CAP chemotherapy (cisplatin, doxorubicin, cyclophosphamide) with complete remission on CT and PET. However, bilateral pleural disease recurred in March 2005, and he received four cycles of ChIVPP chemotherapy (chlorambucil, vinblastine, procarbazine and prednisolone) and radiotherapy to the left chest wall and diaphragm. Residual disease was seen on CT and PET and he pursued non-conventional therapies over the next 12 months.

Upon return in September 2006, he had multiple new pulmonary nodules and progressive pleural disease. In June 2007 there was significant pulmonary and pleural progression, and he was enrolled onto a phase IB study of motesanib diphosphate (AMG 706) in advanced solid tumours. Motesanib diphosphate is a novel oral, highly specific inhibitor of vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, VEGFR-3, Kit and platelet-derived growth factor receptor (PDGFR).

The patient received 75 mg of motesanib diphosphate twice daily in three week cycles (two weeks on treatment, one week off). He had stable disease by RECIST (Response Evaluation Criteria in Solid Tumours), although there was a modest increase in tumour measurements that reached a maximum after 11 cycles (January 2008). Subsequently there

Correspondence: Arun Azad, Medical Oncology Research Fellow, Medical Oncology Unit, Level 6, Harold Stokes Building, Austin Hospital, 145–163 Studley Rd, Heidelberg, Victoria, Australia, 3084. Tel: +61 3 9496 5000. Fax: +61 3 9457 6698. E-mail: arun_azad@hotmail.com

*Joint senior authors

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