REVIEW OF CARCINOMA ANTIGEN 125 (CA 125) AS A MARKER OF DISEASE PROGRESSION OR RECURRENCE IN CLINICAL TRIALS

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Summary

This paper reviews the history, advantages, validity, optimal use, and limitations of the use of CA 125 for assessing the disease response and progression in clinical trials for ovarian cancer patients. The review also explains how, in certain circumstances, RECIST has its own limitations and can be replaced with CA 125. Literature search was conducted in Pubmed and Google for previously published relevant articles and literature on this topic were reviewed. Keeping limitations in view, CA 125 can be used for response assessment in relapsed ovarian cancer trials and for progression assessment in first line ovarian cancer trials. It is concluded that CA 125, being a surrogate of RECIST, should be used in discussion with regulatory agencies in phase II trials to guide for go-no go decision for further phase III clinical development. It is also recommended that the response and progression data should be provided to GCIG for further and prospective validation of the use of CA 125 for response and progression assessment.

Keywords: Carcinoma Antigen-125; Ovarian cancer; Tumor marker; Surrogate marker

Introduction

Measuring disease response to the given therapy in cancer clinical trials serves as indicator of therapy effectiveness. This information is used to decide whether to continue, change or stop the therapy and also used by regulatory agencies for the purpose of approval. The Response Evaluation Criteria in Solid Tumors (RECIST) [1, 2] have been accepted as the standard criteria for measuring response in solid tumors. For most ovarian cancer patients, debulking surgery followed by chemotherapy (CT) remains the treatment of choice. After initial surgery, patients may have no macroscopic disease or patients may present with widespread diffuse peritoneal disease, difficult to measure on CT scans. In such cases the response cannot often be measured using RECIST criteria. [3, 4] Tumor marker CA 125 has emerged as surrogate of RECIST for assessment of treatment response and disease progression in ovarian cancer trials especially where the disease is not measurable by RECIST. The advantage, then, of measuring response and progression according to CA 125 is that more patients who were previously ineligible under RECIST criteria are now eligible for entry into clinical trials, utilizing the patient resources to maximum.

CA 125 is a glycoprotein found in blood and commonly referred as "biomarker" or "tumor marker" for ovarian cancer. [5, 6, 7] The ovarian cancer mucin, CA125, was first identified by the monoclonal antibody, OC125, in 1981. [8, 9] and its genetic structure has been determined recently. [10, 11] CA125 molecule is composed of a short cytoplasmic tail, a transmembrane domain, and an exceptionally large glycosylated extracellular domain dominated by in excess of 60, 156-amino-acid repeat units known to bind the antibodies OC125 and M11. This is present within normal ovarian tissue and on the epithelium of endometrium, endocervix, and fallopian tubes. [4]

CA 125: DEVELOPMENTAL HISTORY

Based on early experience with immune therapy for cancer, investigators started searching for something unique on the surfaces of ovarian cancer cells that could be used to trigger recognition of tumor cells by the immune system. After 125 attempts, an antibody was found. The antibody was termed OC-125 (for the 125th antibody tested against ovarian cancer) and recognized a tumor cell surface antigen termed CA 125. Unfortunately, attempts to use this antibody in treatment were not successful. However, researchers recognized an interesting phenomenon about the protein (antigen) and antibody they were testing – the antigen blood levels seemed to correlate with the status of the ovarian cancer. New studies were launched to see if CA 125 might be useful to diagnose and follow ovarian cancer patients. Early studies identified that as many as 85% of ovarian cancer patients have elevated values. [5] Studies have shown serum CA 125 levels to be elevated in more than 90% of patients with advanced ovarian cancer. [8, 13] CA 125 test reflects the amount of protein released into the blood stream. For adults, considered normal limit of CA 125 test is 0-35 units/mL (0-35 kilounits)/L [12] with 99% of healthy women having values <35 units. Levels above 35 units are certainly seen in healthy women, but beyond the cutoff point of 35, higher the value, more likely there is a pathology somewhere in the body. Women with ovarian cancer often have levels measured in hundreds and even thousands of units

CA 125 AND FALSE ELEVATION

Elevated CA 125 levels do not always indicate ovarian cancer and can be misleading. For instance, CA 125 can be absent when disease is present (false negative), or levels can be high when no disease exists (false positive). Normal tissues, including ovarian cells, pancreatic and breast cells, and the tissues lining the abdomen and chest all make and release low levels of CA 125. Ovarian cancer not only increases the number of cells that make CA 125, but also inflames the abdominal linings that make and release this biomarker. So, it's not surprising that CA 125 is elevated in ovarian cancer and in some other abdominal cancers. Non-cancerous conditions can also elevate CA 125 levels, such as inflammatory conditions of the abdomen (diverticulitis, peritonitis, pelvic inflammatory disease, IBD, tuberculosis and pancreatitis), liver disease, recent surgery, and benign gynecologic conditions such as fibroids, endometriosis, ectopic pregnancy, or a ruptured cyst. Karen *et al.*. discuss how CA125 in combination with other tests can be used in the differential diagnosis of pelvic masses and as part of the investigations for cancer screening. [6] Studies have shown elevated CA125 in 40% of patients with any primary cancer with extensive intra-abdominal disease.[4] Therefore these other diagnoses must be considered in the interpretation of elevated CA 125 value.

VALIDITY/ VALIDATION OF CA 125 CRITERIA

Accepting CA 125 as a surrogate of RECIST, validation is importantly requires indicating that, up to the acceptable limits, it truly measures the state of ovarian cancer and results are reliable. Simplest method used to validate response based on CA 125 has been to compare response rates according to CA 125 with response rates according to standard criteria and calculate the proportion of patients in whom the CA 125 prediction agrees or differs with the response determined by standard criteria. [14] There are difficulties in validating a new response criteria based on a tumor marker, as some patients are only assessable by scans and some are only assessable by the tumor marker and can not be compared directly. In some patients, scans might classify the response differently from the tumor marker. For example, if a patient is classified as having stable disease by scans but as a responder according to the tumor marker, which is correct?

The accuracy of the response according to CA 125 has been determined by examining how accurate the CA 125 defined response was in predicting the activity of drugs in phase II trials, compared with response rates obtained by standard criteria.[15] Retrospective analysis of response data from 19 phase II clinical trials with 14 different cytotoxic drugs for relapsed ovarian cancer showed that there was no statistically significant difference between response rates obtained by standard and CA125 criteria and, therefore, there was no difference in predicting whether a phase II drug was active and worth pursuing in further clinical trials. Response rates were estimated in 1,457 assessable patients according to standard criteria and in 1,092 assessable patients according to CA12.[4]

It is also important to determine which response criterion is the more reliable method for predicting survival and clinical benefit. Gronlund *et al.* [16] retrospectively validate the CA 125 response criteria in 131 patients who received second-line CT by comparing the prognostic value of response by the CA 125 definition with response by the RECIST criteria. They found that the CA 125 criteria was 2.6 times more accurate than RECIST at

predicting survival. Although on univariate analysis both RECIST and CA 125 responses were each significantly correlated with survival and in a Cox analysis, only the CA 125 response was significant.

USE OF CA 125 IN OVARIAN CANCER TRIALS

In ovarian cancer clinical trials CA 125 is primarily used for two purposes: outcome prediction (response evaluation) and detection or assessment of disease progression. GCIG (Gynecologic Cancer InterGroup) has been pioneer in evaluating CA 125 uses. GCIG consists of representatives from the major gynecologic cancer trial groups around the world. An extensive work on validation of CA 125 criteria for response and progression assessment in ovarian cancer trials has been done by GCIG. Uses of CA 125 criteria as per the GCIG recommendations are provided in table 1. [17] GCIG recommends that definitions for response in relapse trials and progression in first line trials according to serum CA 125 levels should be incorporated into ovarian cancer clinical trial protocols. [18] For patients who do not have elevated CA 125 levels or whose levels have neither responded nor progressed by the GCIG criteria, response and clinical benefit must be assessed by standard methods. [4]

Table 1. GCIG recommendations for response and progression assessment by CA 125

	Use Recommended by GCIG	Not standard and Needs further validation	Not recommended by GCIG
Evaluation of response	Relapse Trials	Maintenance or Consolidation Trials	First Line Trials
Assessment of progression	Front Line Trials	Maintenance or Consolidation Trials and Relapse Trials	-

EVALUATION OF 'RESPONSE' IN OVARIAN CANCER TRIALS

Evaluation of disease response can be done with CA 125 criteria especially in situations where the response can not be assessed with standard RECIST or patient presents with widespread diffuse peritoneal disease and ascites. The 'response evaluation' with CA 125 is validated and recommended by GCIG in clinical trials only of relapse ovarian cancer whereas in maintenance or consolidation and first line therapy clinical trials the use of CA 125 is not standard and there is no data to validate response evaluation in this situation and needs further validation.

Evaluation of response in relapsed ovarian cancer trials

Several different definitions of response based on CA 125 have been proposed earlier, which has led to a variation in the response rate from 10% to 62% in the same group of patients, depending on the used definition. [19] On the basis of available data and extensive discussions and debate, GCIG has proposed precise but simple definition, given below (Figure 1.) for response assessment by CA 125 [3] and recommends its use so that response can be measured by either RECIST or CA 125 criteria or in combination. [17, 18]

"A response according to CA 125 has occurred if there is at least a 50% reduction in CA 125 levels from a pretreatment sample. The response must be confirmed and maintained for at least 28 days. Patients can be evaluated according to CA 125 only if they have a pretreatment sample that is at least twice the upper limit of normal and within 2 weeks prior to starting treatment."

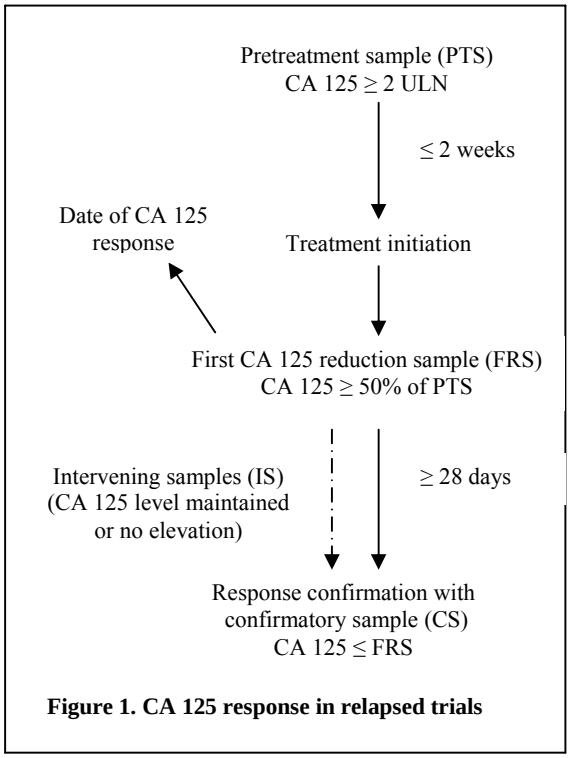

Figure 1. CA 125 response in relapsed trials

The date when the CA 125 level is first reduced by 50% is the date of the CA 125 response. If the response is evaluable by both CA 125 and RECIST, then the date of response will be the date of the earlier of the two events. [17, 18] To calculate CA 125 responses accurately, following rules apply:

1) Intervening samples and the 28-day confirmatory sample must be less than or equal to (within an assay variability of 10%) the previous sample.
2) Variations within the normal range of CA 125 levels will not interfere with the response definition.
3) For each patient, the same assay method must be used and the assay must be tested in a quality-control scheme.
4) Patients are not evaluable by CA 125 if they have received mouse antibodies (unless the assay used has been shown not to be influenced by HAMA [20, 21] or if there has been medical and/or surgical interference with their peritoneum or pleura during the previous 28 days.

GCIG recommends that, (1) ideally, the CA 125 measurements to be taken at specific time intervals, (2) the first sample would be collected within 2 weeks before treatment initiation and (3) later samples would be collected at intervals of 2–4 weeks during treatment and at intervals of every 2 or 3 months during follow-up. [18]

Considerations for trial design and response analysis

If therapy includes two treatment modalities (e.g., surgery and CT), and CA 125 response results from both treatments, then it should be clearly stated that CA 125 cannot distinguish between the effects of each treatment. [17, 18]

To calculate response rates in trials, an intent-to-treat analysis should be used that includes all patients with an initial CA 125 level of at least twice the upper limit of normal (ULN) as eligible and evaluable. In addition, as a separate analysis, those patients who have both a CA 125 response and whose CA 125 level falls to within the normal range, can be classified as complete responders. [22] Patients who have a fall of CA 125 to within the normal range but whose initial CA 125 was <2ULN, have not had a CA 125 response and cannot therefore be classified as a CA 125 complete responder - Figure 2. [17, 18]

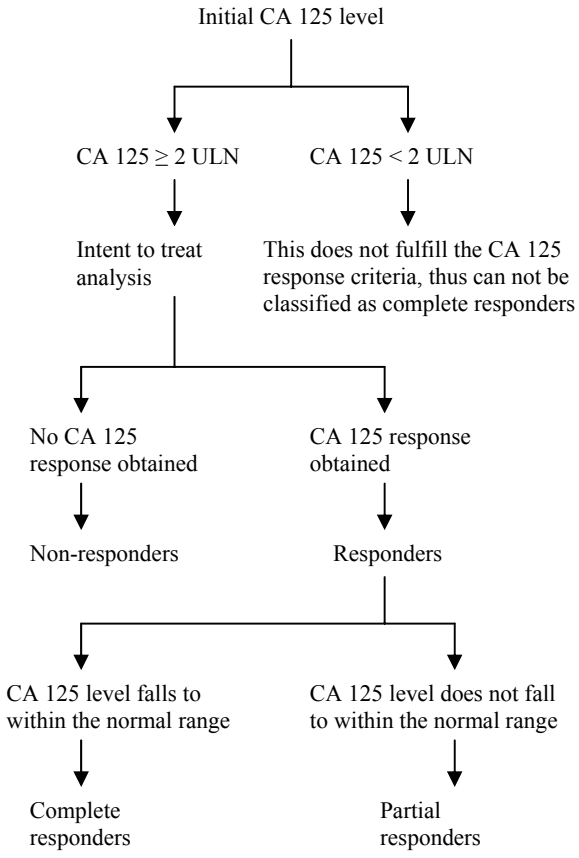

Figure 2. Considerations for CA 125 responders

Evaluation of response in maintenance or consolidation trials

For maintenance or consolidation trials response in patients whose initial CA 125 is >2ULN can be evaluated according to the GCIG CA 125 response definition. It should be noted that this is not a standard use of CA 125 criteria and there is no data to validate response evaluation in this situation and needs further validation. To prevent the prior therapy interfering with the response assessment the following requirement is recommended. [17]

- Two pre-treatment samples no more than 8 weeks apart are required if test treatment is given as part of maintenance or consolidation therapy.
- For the test treatment to be evaluable according to CA 125 there must be no more than a 10% fall in CA125 between the two pretreatment samples.
- The sample closest in time to the test therapy should be considered the pre-treatment sample.

Evaluation of response in first line trials

The CA 125 response definition has been produced to evaluate response in relapse therapy. [18] It should be noted that there is no data to validate response evaluation in trials where patients receiving first line therapy and this use is not recommended by GCIG. It should be remembered that for a patient to be classified as a complete responder according to RECIST, tumor marker levels such as CA 125 must be within the normal range. [17]

ASSESSMENT OF PROGRESSION IN OVARIAN CANCER TRIALS

Assessment of disease progression can be done with CA 125 criteria for progression; however it is recommended by GCIG only in first line clinical trials whereas in relapse and maintenance or consolidation clinical trials its use is not standard and there is no data to validate progression assessment in these situations and needs further validation.

Assessment of progression in first line therapy studies

Progression can also be measured based upon serum CA 125 but tumor measurements should take precedence over CA 125. GCIG has developed definitions of CA 125

progression to complement the definitions of objective disease progression for use in first-line CT trials in ovarian cancer. If measurable disease is shrinking during treatment, but the CA 125 indicates progression the patient should continue to receive protocol treatment. If measurable disease shows stable disease but CA 125 indicates progression after a minimum of 3 courses of CT, protocol treatment should be changed. [17]

The definitions of CA 125 progression are based on the well known and validated definitions of progression in ovarian cancer using serum CA 125 levels. [23] The published data support the concept that, after first-line therapy, doubling in CA 125 from the ULN reliably predicts objective progression. For those patients whose CA 125 never fell to the normal range, a doubling from the nadir has been shown to predict progression. [24] The proposed definitions of progression in Table 2. consider three patient groups, according to their serum CA 125 behavior. Progression based on serum CA 125 levels will be defined on the basis of a progressive serial elevation of serum CA 125, according to the following criteria:

A. Patients with elevated CA 125 pretreatment and normalization of CA 125 must show evidence of CA 125 ≥ 2ULN on two occasions at least one week apart;
B. Patients with elevated CA 125 pretreatment, which never normalizes must show evidence of CA 125 ≥ 2 times the nadir value on two occasions at least one week apart;
C. Patients with CA 125 in the normal range pretreatment must show evidence of CA 125 ≥ 2ULN on two occasions at least one week apart.

Elevated values must be confirmed by two separate measurements obtained at least one week apart. CA 125 progression will be assigned the date of the first measurement that meets the criteria as noted. Patients are not evaluable by CA 125 if they have received mouse antibodies (unless the assay used has been shown not to be influenced by HAMA) [20, 21] or if there has been medical and/or surgical interference with their peritoneum or pleura during the previous 28 days.

It should be emphasized that these definitions of progression are intended for the specific context of first-line therapy studies. A patient may be declared to have progressive disease on the basis of either the objective RECIST or the CA 125 criteria. The date of progression will be the date of the earlier of the two events if both are documented. [17, 24]

Table 2. Definition of progression after first-line therapy in ovarian cancer

	Categorized Patient groups		
	A	B	C
RECIST – Measurable or non-measurable disease	Compared to baseline (or lowest sum while on study if less than baseline), a 20% increase in sum of longest diameters (RECIST definition; *1*) or Any new lesions (measurable or nonmeasurable) Date PD: date of documentation of increase or new lesions		
	and/or†		
	A	B	C

CA 125	CA 125 ≥ 2× ULN documented on two occasions‡ Date PD: first date of the CA 125 elevation to ≥ 2× ULN	CA 125 ≥ 2× nadir value on two occasions‡ Date PD: first date of the CA 125 elevation to ≥ 2× nadir value	As for A

ULN = upper limit of normal; PD = progressive disease.
†A. Patients with elevated CA 125 pretreatment and normalization of CA 125; B. Patients with elevated CA 125 pretreatment, which never normalizes ; C. Patients with CA 125 in normal range pretreatment.
‡Repeat CA 125 any time, but normally not less than 1 week after the first elevated level. CA 125 levels sampled within 4 weeks after surgery, paracentesis, or administration of mouse antibodies should not be taken into account**.** [24]

Since it was recognized that the timing of investigations during first-line therapy and subsequent follow-up may also influence the date of progression free survival in clinical trials, it is recommended that the serum CA 125 levels be obtained on day 1 of each chemotherapy cycle, 4 weeks after the last course, thereafter every 3–4 months for the first 36 months, every 6 months from month 37–60, and every year from 5 years after the primary diagnosis. [5]

Assessment of progression in relapse and maintenance or consolidation clinical trials
The CA 125 definition for assessment of progression has been produced to evaluate progression in first line therapy. If the GCIG definition based on CA 125 is used to define progression after relapse or maintenance or consolidation therapy it should be noted that it has not been validated and is not recommended by GCIG.

USE OF CA125 CRITERIA IN FUTURE CLINICAL TRIALS

Therasse *et al.* [1] discussed the role of combining tumor marker response definitions with the RECIST criteria in future clinical trials. In the case of ovarian cancer trials, this has the advantage of evaluating more patients according to CA125 or RECIST or by both criteria. Eligibility for trials in which response rate is an end point could be broadened to include either RECIST or CA 125 assessable patients, as many patients cannot be adequately assessed by conventional imaging techniques, but might well have elevated CA 125 levels. Trials could be designed with a 90% power to detect the minimal acceptable rate in either the standard RECIST or the CA 125 response, greatly saving patient resources. [3]

It may not be possible to get fast-track regulatory approval based on CA 125 response alone because of the possibility that a novel agent could interfere with CA 125 synthesis or release. There have been reports of unreliable CA 125 measurements after paclitaxel therapy, but this has not been corroborated in other studies in which a precise definition for CA 125 response was used. [25-27] However, the CA125 response and progression criteria were derived from known data sets and have been retrospectively validated. Therefore, at present, in order to use CA125 criteria as a secondary end point in clinical trial design, it should be discussed with the regulatory authorities before incorporating in a trial protocol.

The GCIG is very keen that CA125 response definition be tested in a wide variety of new phase II trials to facilitate prospective validation and general acceptance. [4]. The CA 125 response definition should be used to support so-called go/no-go decisions for further development of drugs in phase II trials. If CA125 response rates are lower than a predetermined threshold efficacy, the drug should be rejected and further studies are not necessary. However, if CA125 response rates are satisfactory, the patient numbers within the trial should be expanded allowing sufficient patients to be evaluated by both CA125 and RECIST criteria. For patients who do not have elevated CA 125 levels or whose levels have neither responded nor progressed by the GCIG criteria, response and clinical benefit must be assessed by standard methods.[4] Examples of the use of the CA125 response and progression definitions in recently published data include the phase II trials for oral altretamine in relapsed ovarian carcinoma[28] and the use of weekly cisplatin and oral etoposide in relapsed ovarian cancer.[29]

If a patient has received human antimouse antibody therapy, the assay may become unreliable, although there are ways to overcome this problem.[30] It is important to recognize other limitations of CA 125. Levels can be altered dramatically by abdominal surgery or peritonitis. There is also the possibility of laboratory error and considerable variation in results among laboratories. Despite these cautions, increased confidence in a CA 125 response definition should lead to a cheaper and, in some cases, more accurate method for monitoring ovarian carcinoma therapy than standard radiographic criteria.

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