A critical review of the analytical approaches for circulating tumor biomarker kinetics during treatment

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Changes in serum tumor biomarkers may indicate treatment efficacy. Traditional tumor markers may soon be replaced by novel serum biomarkers, such as circulating tumor cells (CTCs) or circulating tumor nucleic acids. Given their promising predictive values, studies of their kinetics are warranted. Many methodologies meant to assess kinetics of traditional marker kinetics during anticaner treatment have been reported. Here, we review the methodologies, the advantages and the limitations of the analytical approaches reported in the literature. Strategies based on a single time point were first used (baseline value, normalization, nadir, threshold at a time t), followed by approaches based on two or more time points [slope, rate of change, time-to-events...]. Heterogeneities in methodologies and lack of consideration of inter- and intra-individual variability may account for the inconsistencies and the poor utility in routine. More recently, strategies based on a population kinetics approach and mathematical modeling have been reported. The identification of equations describing individual kinetic profiles of biomarkers may be an alternative strategy despite its complexity and higher number of necessary measurements. Validation studies are required. Efforts should be made to standardize biomarker kinetic analysis methodologies to ensure the optimized development of novel serum biomarkers and avoid the pitfalls of traditional markers.

Key words: tumor biomarkers, kinetics, predictive factor, prostate adenoma, prostate cancer, tumor biomarkers

introduction

Circulating tumor biomarkers are molecules produced by cancer cells or released by an organism in response to the presence of cancer \cite{1, 2}. Parallel changes in serum tumor biomarker titers and cancer growth have been reported in many cancers \cite{2}. As a result, the kinetics of circulating tumor biomarker titers during treatment, considered to be an early reflection of treatment efficacy, has been extensively investigated. The need for prognostic factors able to predict treatment efficacy and the risk of relapse/progression before imaging results have urged the development of such studies.

Modern serum biomarkers, such as circulating tumor cells (CTCs) and circulating tumor nucleic acids, are emerging as novel prognostic or predictive factors \cite{2–5}. As illustrated by the promising prognostic values of early decrease in CTCs in metastatic prostate cancer patients \cite{6, 7} or circulating tumor nucleic acids in colorectal cancer patients \cite{8}, the development of these novel tumor biomarkers shall require investigation of their kinetics during treatment. Scientists and clinicians involved in the development of these biomarkers will be confronted with the challenge of selecting the best analytical methods among dozens that have been reported in the literature. Indeed, many approaches meant to assess the time-related changes of traditional serum tumor marker titers and their prognostic values have been reported, but the optimal methodology has not been consensually determined. The experience gained with traditional biomarker studies may help define strategies that should be abandoned or others that should be explored for the analysis of novel serum tumor biomarker kinetics in the future.

methods

The main objective of the present analytical study was to review the methodologies, the strengths and the limitations of the different approaches that have been used to assess the kinetics of serum tumor biomarkers during treatment along with their potential prognostic or predictive values regarding treatment efficacy. The role of kinetic biomarkers in cancer screening or diagnosis was not assessed in the present study.

A literature search was conducted for the different methodologies used to analyze serum tumor biomarker kinetics during treatment using PubMed (www.pubmed.org) (see supplementary Appendix, available at Annals of Oncology online).
approaches used to analyze tumor biomarker kinetics during anticancer treatment

The results of the present analysis are summarized in Tables 1 and 2 (supplementary Appendix, available at Annals of Oncology online).

approaches based on the titer of a single time point

The identification of a specific predictive time point that discriminates between patients with favorable and unfavorable tumor biomarker decline curves has been a common strategy. This approach offers the advantage of simplicity, as it can be implemented easily by investigators involved in clinical trials or assessed by clinicians in routine practice.

baseline (pre-treatment) concentration

The predictive or prognostic values of the baseline concentrations, measured before initiating treatment, have been described for many tumor biomarkers (Figure 1A). These studies involved CEA in colorectal cancers [9, 10], CA125 in ovarian cancers [11–13], AFP in hepatocellular carcinoma [14, 15] and germ cell tumors, CA15–3 in breast cancers [16–18], prostate specific antigen (PSA) in prostate cancers [19–21], and CA19–9 in colorectal cancer [22, 23] and pancreatic cancers [24–27] among others. Of them, the baseline serum concentrations of hCG and AFP in germ cell tumors, hCG in trophoblastic tumors and PSA in prostate cancer are routinely used for adjusting treatment–tumor biomarker decline curves has been a common strategy. This approach offers the advantage of simplicity, as it can be implemented easily by investigators involved in clinical trials or assessed by clinicians in routine practice.

normalization of tumor biomarkers

According to this strategy, the tumor biomarker titer is supposed to be in the normal range at a time t. Conversely, an abnormal value at time t means treatment failure and a higher risk of relapse (Figure 1B). This method easily manageable by investigators and clinicians implies that the normalization is expected, and the observation time-frame has been determined.

This approach has been used primarily for the analysis of CA125 kinetics in patients with gynecological cancer treated with chemotherapy. In patients with metastatic ovarian cancer, CA125 normalization at the end of six chemotherapy cycles was reported as a predictive factor in a review of seven Gynecology Oncology Group trials [47] or during the third cycle in two other studies [48, 49]. Similar conclusions were drawn about the value of CA125 normalization after neoadjuvant chemotherapy in patients with advanced ovarian cancer [50, 51] or after three chemotherapy cycles in patients with metastatic endometrial cancer [52].

The predictive values of tumor biomarker normalization were also reported with PSA in metastatic prostate cancer patients treated with hormone treatment [30], CA19–9 in pancreatic cancer patients treated with surgery [30], CA15–3 in breast cancer patients [53], CEA, CA19–9 and CA125 in non-small-cell lung cancer patients [54], hCG in gestational trophoblastic disease [55] and squamous cell carcinoma in cervical carcinoma patients, all treated with chemotherapy [56].

Of note, the kinetics of CTCs were investigated in patients with metastatic prostate and colorectal cancer patients. It was suggested that a static CTC cut-off is the best method to determine whether a therapy is effective. Elimination of all CTCs offered the best prognostic in terms of overall survival [36]. The same conclusions were drawn in breast cancer patients treated with neoadjuvant chemotherapy [57].

As illustrated by the poor utility of this method in research or in routine despite extensive publications, except for hCG in gestational trophoblastic disease [55], this approach is limited by the requirement of a normalization time frame, and the paucity of tumor biomarker normalization for most of metastatic diseases.

nadir

The minimum titer observed at time t, which is frequently at the end of treatment, is considered a predictive factor for treatment efficacy (Figure 1C). This measure has been mainly used to analyze the kinetics of CA125 in ovarian cancer patients [58–61], CA 19-9 in pancreatic cancer patients, CEA in colorectal cancer patients and PSA in prostate cancer patients. However, the reported thresholds have been inconsistent among studies for the same biomarkers. For example, different PSA nadir predictors of relapse risk have been reported in prostate cancer patients treated with radiotherapy (0.5 ng/ml [62, 63], 0.2 ng/ml [64], 1.2 ng/ml [65] or 1.5 ng/ml [66]), radical prostatectomy (no cut-off due to continuous predictive value [67], 0.4 ng/ml [68], 0.01 ng/ml [69]) or hormone therapy (0.1 ng/ml [70, 71], 0.2 ng/ml [72, 73], 0.4 ng/ml [74] or 0.5 ng/ml [75]).

As a consequence of these limitations, this methodology is not used in routine practice to assess treatment efficacy in research or in routine.

cut-off at time t

A threshold different from the assay normal limit or nadir, measured at time t, is used as a predictive factor (Figure 1D). Predictive cut-offs were reported for PSA in patients with prostate cancer after surgery [76], CA125 in patients with ovarian cancer [60, 77], CA19–9 in patients with pancreatic cancer [78, 79] or hCG in patients with trophoblastic disease [80], all treated with palliative chemotherapies. However, the reported cut-offs were heterogeneous. For example, different thresholds were reported for hCG titers regarding the risk of relapse or...
### Table 1. Summary of the advantages, limitations and utility of approaches based on a single, or minimum two time points

<table>
<thead>
<tr>
<th>Methodologies</th>
<th>Principles</th>
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<th>Specific limitations</th>
<th>Utility as stated by expert consensus</th>
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<tr>
<td>Approaches based on a single time point (Figure 1)</td>
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<td>Easy assessment</td>
<td>No integration of subsequent tumor marker values</td>
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<tr>
<td><strong>General advantages:</strong></td>
<td></td>
<td></td>
<td></td>
<td>(i) hCG anf AFP in NSGCT [28]</td>
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<td>(ii) hCG in gestational trophoblastic neoplasia [29]</td>
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<td>(iii) PSA in prostate cancer [30]</td>
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<td>(i) Use of a unique time point to characterize the complex kinetics of tumor markers</td>
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<td>(ii) Impact of inter- and intra-individual variability of assays on outcomes</td>
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<tr>
<td>Normalization of tumor markers</td>
<td>Predictive value of tumor marker titers in the normal range at time t</td>
<td>Intuitive favorable predictive value of tumor marker titers normalization</td>
<td>(i) Determination of tumor marker normalization time frame required</td>
<td>hCG in gestational trophoblastic neoplasia [41]</td>
</tr>
<tr>
<td>Nadir</td>
<td>Predictive value of the minimum value of tumor marker kinetics during an observation time frame</td>
<td>Assumption about links between tumor marker concentrations and tumor size</td>
<td>(i) Determination of tumor marker observation time frame required</td>
<td>None</td>
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<tr>
<td>Cut-off at a time t</td>
<td>Predictive value of a threshold at a time t</td>
<td>Easy assessment</td>
<td>Inconsistencies in methods used for defining a predictive reproducible threshold</td>
<td>None</td>
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<tr>
<th>Methodologies</th>
<th>Principles</th>
<th>Specific advantages</th>
<th>Specific limitations</th>
<th>Utility as stated by expert consensus</th>
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<td>Intuitive predictive value of percentage reduction</td>
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<td>PSA response in metastatic prostate cancer [84]</td>
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<tr>
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<td>General advantages</td>
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<td>(ii) Impact of selected time points on outcomes</td>
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<td>(ii) Reduced number of samples</td>
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<td>General limitations:</td>
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<td></td>
<td>(i) Impact of inter- and intra-individual variability of assays on outcomes</td>
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<td></td>
<td>(ii) Simplification of the complex kinetics of tumor markers</td>
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<tr>
<td>Decline slope</td>
<td>Predictive value of tumor marker reduction</td>
<td>Similarity with mathematical concepts</td>
<td>Lack of intuitive predictive value of the slope</td>
<td>None</td>
</tr>
<tr>
<td>HL</td>
<td>Predictive value of the time to 50% decrease of tumor marker titers</td>
<td>Similarity with drug concentration decline in pharmacokinetic studies</td>
<td>(i) Assumptions about kinetic profiles of tumor marker kinetics required: maximum one or two HLs (ii) Impact of selected time points on outcomes (iii) Inconsistencies in methods used for defining a predictive reproducible threshold</td>
<td>None</td>
</tr>
<tr>
<td>Time to nadir</td>
<td>Predictive value of the time to tumor marker minimum value</td>
<td>Assumption about links between tumor marker concentrations and tumor size</td>
<td>Repetitive measurements of tumor marker concentrations required</td>
<td>None</td>
</tr>
<tr>
<td>TTN</td>
<td>Predictive value of time to tumor marker normalization</td>
<td>Intuitive favorable predictive value of tumor marker titer normalization</td>
<td>Normalization not necessarily expected in metastatic disease</td>
<td>None</td>
</tr>
<tr>
<td>AUC</td>
<td>Predictive value of the area under the tumor marker concentration versus time curve</td>
<td>Similarity with drug concentration decline in pharmacokinetic studies</td>
<td>Complexity of calculation</td>
<td>None</td>
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</tbody>
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Table 2. Summary of the advantages, limitations and utility of model-based approaches

<table>
<thead>
<tr>
<th>Methodologies</th>
<th>Principles</th>
<th>Specific advantages</th>
<th>Specific limitations</th>
<th>Utility as stated by expert consensus</th>
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<tr>
<td>Model-based approaches</td>
<td>Empirical models (Figure 3)</td>
<td>Determination of the mathematical equations using pharmacokinetic principles (e.g. bi-compartment clearance-based model)</td>
<td>(i) Similarity with drug concentration decline in pharmacokinetic studies (ii) Easy determination of potential predictive kinetic parameter (e.g. clearance)</td>
<td>None</td>
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<tr>
<td>General advantages:</td>
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<td>(i) Dynamic analysis of tumor marker kinetics (e.g. determination of the individual mathematical equations)</td>
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<td>(ii) Reduced impact of inter- and intra-individual variability of assays on outcomes</td>
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<td>(iii) Quantification of inter- and intra-individual variability</td>
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<tr>
<td>General limitations:</td>
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<td>(i) Complexity of model building</td>
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<td>(ii) Minimum three to four time points per patient required</td>
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<tr>
<td>Mechanistic models (see supplementary Figure 4, available at Annals of Oncology online)</td>
<td>Sets of equations to describe tumor maker production dependently on tumor size &amp; growth, treatment dose &amp; dosing schedule and marker elimination</td>
<td>(i) Utility for understanding the effects of treatments on tumor marker production and on tumor size (ii) Early prediction of treatment efficacy or of resectability (iii) Potential utility for drug development</td>
<td>(i) Complexity of model building (ii) Mathematical, statistical and computer processing skills required</td>
<td>None</td>
</tr>
</tbody>
</table>
chboro-lesion during the seventh week of methotrexate treatment in patients with low-risk trophoblastic tumors (56 µg/l [81], 737 IU/l [82] or 500 mIU/ml [83]).

In addition to the inconsistency of cut-off values, discrepancies exist in the methods identifying predictive factors (see supplementary Appendix, available at Annals of Oncology online) [60, 76–78, 81, 82, 84].

Despite extensive reports, none of the reported predictive cut-offs have been validated for research purposes or used in routine practice for treatment adjustment. This method integrates strong limitations able to reduce its relevance for development of novel tumor biomarkers. This approach has also been selected for analyzing the predictive value of CTC kinetics in patients with metastatic prostate cancer, colorectal cancer, breast cancer and non-small cell lung cancers with heterogeneous predictive cut-offs [6, 31, 33, 36, 85–89].

**Figure 1.** Illustrations of four single time point approaches frequently used to analyze serum tumor biomarker kinetics. Serum tumor biomarker concentrations (light grey dots) are plotted versus time in a typical patient. (A) Baseline titer. (B) Normalization of the tumor biomarker at time \( t \). (C) Nadir during the period of observation. (D) Cut-off at time \( t \).

**limitations of approaches based on a single time point**

In addition to specific limitations related to each method, the strategies based on a single time point have a major general limitation which is the use of a unique titer value to characterize the complex kinetics of tumor biomarkers. This might lead to inaccuracy, as they rely on a single concentration at which the measurement is prone to inter- and intra-individual variability [2, 90]. For example, a single time point outside the normal decline curve might lead to wrong conclusions about an abnormal decrease or increase.

**kinetic approaches based on a minimum of two time points**

The analysis of two or more time points to describe decrease curves for serum tumor biomarkers during treatment is rational. Indeed, this strategy is less dependent on the inter- or intra-individual variability of measurements than a single time point-based approach.

**percentage change in tumor biomarker titer concentrations**

The decrease in tumor biomarker percentage is assessed with or without a time frame. A predictive percentage cut-off capable of
discriminating between patients with favorable and unfavorable declines is generally sought (Figure 2A). The predictive values of decreases in tumor biomarker percentages were reported for CA125 in metastatic ovarian cancer patients [91–94], CEA, CA15-3 in patients with breast cancer [95–97], CA19-9 in advanced pancreatic cancer patients treated with chemotherapy [98, 99] and more recently for circulating tumor nucleic acids in patients with metastatic colorectal cancer [8].

In 1999, following several studies demonstrating that a decrease of PSA ≥50% over an 8- to 12-week treatment period was associated with longer median survivals in castration-resistant prostate cancer patients treated with systemic treatments [100–102], the PSA working group declared a PSA decline ≥50% with two measurements 3–4 weeks apart was the official definition of a biochemical PSA response in clinical trials [103, 104]. Since that time, this criterion has been widely used to assess the efficacy of systemic treatments [105–110], though its prognostic value has been called into question in two recent phase III trials in which a ≥30% decline was reported as a better predictive factor [30].

In 2004, the Gynecologic Cancer Intergroup defined a CA125 response to treatment as a 50% reduction in CA125 levels maintained for at least 28 days [111]. However, the predictive value of this kinetic parameter has been reconsidered after the retrospective CALYPSO trial, which demonstrated an early decline in CA125, and that the response was more frequent in the detrimental arm [112].

The percentage decrease is a popular kinetic parameter that has been frequently reported for research purposes, as a surrogate biomarker of treatment efficacy. This kinetic parameter has been adopted by international expert groups for two serum tumor biomarkers. However, the reproducibility of the surrogate values of these parameters tends to be reconsidered in recent large studies.

decline slope

The predictive value of decreased slope, generally calculated using linear regression, has been poorly reported (Figure 2B). With the exception of the predictive values of the decline in the CA15-3 slope in patients with metastatic breast cancer [113] and CA125 in patients with ovarian cancer treated with chemotherapy [114, 115], this approach has been rarely used.

In a recent study, investigators showed that the trend between two CTC time points had more prognostic value than the absolute value in patients with non-small-cell lung cancer treated with chemotherapy during the first cycle. However, the
methodology used to assess the CTC change was poorly described [116]. The same outcomes were reported for circulating tumor DNA in colorectal cancer or lung patients treated with chemotherapy [42, 45].

**Time-to-event approaches**

These approaches rely on calculating the time required to observe an event, such as

(i) Decrease by 50%, half-life (HL) (Figure 2C)
(ii) Time to nadir
(iii) Time to normalization (TTN) of values

HL is the period of time needed for a biomarker to decrease by 50%. By analogy with pharmacokinetic studies of drugs, HLLs have frequently been used to assess the kinetics of serum tumor biomarkers. According to hypotheses regarding the decline profile of tumor biomarkers, one or two decrease HLLs are calculated (Figure 2C).

For example, the decline in PSA after prostate cancer surgery has been analyzed extensively using HLLs. Inconsistent values for the mono-exponential PSA HLL were reported, ranging from 0.55 to 3.39 days [117–120]. When the PSA decrease after radical prostatectomy was fitted to bi-exponential decline curves, the two HLLs (HL and HL) were reported to have values between 1.4 and 45.4 h and 52.8 and 182.9 h, respectively [118, 121–126]. Similar heterogeneity in the predictive values for the decline in HL was also reported for hCG and AFP in patients with non-seminomatous germ cell tumors (NSGCTs) [127–129], CA125 in patients with ovarian cancer [130] and CEA in patients with colorectal cancers [131–133] all treated with chemotherapy. The predictive values of HLLs were inconsistent across studies, as were the methodologies used to calculate these HLLs (see supplementary Appendix, available at Annals of Oncology online) [117–119, 121, 124–127, 129, 134–147].

TTN is defined as the time required for the normalization of a tumor biomarker titer. TTN has been used mainly to define prognostic groups regarding the risk of progression in NSGCT patients treated with first-line or salvage chemotherapies [148, 149]. The value of TTN in treatment adjustment was recently demonstrated in a GETUG-13 phase III trial [150]. The predictive values of tumor biomarker TTN was also reported for CA125 in ovarian cancer patients treated with surgery and chemotherapy [151, 152] and PSA in patients with prostate cancer treated with radiation [153].

Similarly to HLLs, different methodologies have been used to determine TTN across studies (see supplementary Appendix, available at Annals of Oncology online).

Time to nadir is the time required to observe a decline in tumor biomarkers to the minimum value during a period of observation. The calculation of this kinetic parameter is based on the longitudinal observations of tumor biomarker kinetics. The time to nadir parameter has been investigated mainly for the analysis of PSA in patients with prostate cancer treated with radiation. The results were heterogeneous regarding the predictive value of times to normalization: cut-off at 1 year [154], 2 years [155], 1–3 years [156], no cut-off defined [157, 158] or no predictive value [159–161].

None of the time-to-event parameters are used for treatment adjustment in routine or as surrogate biomarkers in clinical studies although numerous reports have been published. Discrepancies in methodologies and outcomes may explain the poor use of these parameters. Indeed the time points used to assess time-dependent parameters, such as HL, TTN or decline...
slope, have been inconsistent across studies although they are critical for values. For instance, if the actual tumor biomarker decline curve is not mono-exponential, as it seems to be for PSA and CA125, HL outcomes greatly depend on the time points selected for analysis. No guidelines have been defined for the time points selected to assess these kinetic parameters. Consequently, variable time points were chosen and heterogeneous results reported. This inaccuracy might have been increased by the lack of consideration for unexplained inter- and intra-individual variabilities that characterize tumor biomarker values [2, 90]. In addition, most of these approaches are influenced by the period of observation of tumor biomarker values.

**area under the curve (AUC)**

Similar to pharmacokinetic studies, AUC is calculated as the AUC for tumor biomarker concentration versus time during an observational window (Figure 2D). This strategy has been poorly reported (see supplementary Appendix, available at Annals of Oncology online).

**approaches based on mathematical modeling studies**

**non-linear effect models**

Tumor biomarker kinetics can be described using mathematical equations. Model-based strategies are less influenced by the time points selected by investigators and considered more dynamic than previous strategies. Somehow, these strategies are more complex to implement.

Some authors have shown the relevance of non-linear models to describing PSA decline after radical treatments in patients with prostate cancer. Hanlon used non-linear mixed effect modeling to fit PSA decreases after radiation treatment to mono-exponential or bi-exponential models [162]. Ebert et al. recently reported a methodology called the ‘segmented regression process’ to differentiate the PSA kinetics profiles between linear, mono-exponential and bi-exponential decline after external beam radiotherapy [163]. Vollmer built a first-order kinetic semi-mechanistic model using a non-linear least square algorithm to describe the PSA decline after radical prostatectomy and discriminate between PSA produced by benign tissues and PSA released by cancer tissues [120]. Botteli et al. modeled the relationships between CTC kinetics and survival in patients with treated metastatic breast cancer. A nonlinear increase in risk of both progression and death with increasing number of CTCs was observed [164]. Although these models enable the description of the whole tumor biomarker kinetic profile using mathematical equations, no clear predictive kinetic parameters have been reported.

**population kinetics approach and empirical models**

The population kinetics approach is commonly used to analyze the pharmacokinetic parameters of drugs administered to study subjects. The general principles have been described elsewhere [165–167] and are presented in Figure 3. This strategy presents several advantages able to overcome the limitations of other approaches (see supplementary Appendix, available at Annals of Oncology online).

The population kinetics-based approach has been used for the kinetic analysis of several serum tumor biomarkers.
including PSA decline after radical prostatectomy; hCG and AFP in patients with NSGCTs treated with three cycles of BEP regimen and hCG kinetics in patients with low-risk gestational trophoblastic disease treated with methotrexate regimen [168, 169]. In all studies, modeled kinetic parameters with significant prognostic values were identified.

The reproducibility of modeled hCG residual production predictive value was suggested in two large independent cohorts of gestational trophoblastic disease patients [170, 171], as was the validity of modeled PSA clearance as a predictor of relapse after prostatectomy in a recent prospective validation study [168].

mechanistic models

In 1994, Cappelli et al. defined the basic principles of the mechanistic modeling of tumor biomarker kinetics meant to go 'beyond the cut-off'. Potential mathematical models for describing tumor biomarker increases related to tumor growth (logistics models, Gomperz model) or tumor biomarker decreases after radical treatment (non-linear regression models, HLs) [172]. However, these principles did not apply to the analysis of tumor biomarkers.

To describe serum tumor biomarker kinetics more accurately using mathematical models, recognizing the production of tumor biomarkers by cancer dependent on the tumor size, growth and aggressiveness, the impact of treatment dose and dosing schedule on tumor size, and tumor biomarker elimination is necessary. Using the mechanistic modeling approach, tumor biomarker production/elimination related to tumor size and proliferation is described by sets of equations, whereas the pharmacokinetics and effect of chemotherapy on tumor proliferation and biomarker production are described by others.

Such a model was developed for the analysis of CA125 kinetics using the data from CALYPSO phase III trial in which two carboplatin-based regimens were compared in patients with recurrent ovarian carcinoma (see supplementary Figure 4, available at Annals of Oncology online). The authors derived a model-based tumor biomarker elimination parameter, KELIM, which had an independent predictive value regarding [173]. Moreover correlation was subsequently found between the kinetics of CA125 serum titers, tumor size dynamics and survival, which provides a perspective in terms of the prediction of resectability after chemotherapy or of decision making in drug development [174–176]. Validation of these outcomes should be considered in independent cohorts of patients.

Other authors developed mechanistic models to describe tumor biomarker kinetics during treatment. For example, Tanaka et al. used a stochastic model to characterize changes in prostate cancer growth and PSA titer induced by hormone therapy. An individual treatment adjustment strategy was proposed PSA kinetics [177].

If model-based strategies enable the dynamic assessment of serum tumor biomarker kinetics, independent of selected time points, with high accuracy, they are limited by the complexity of the methodology. The implementation of a model requires skills to deal with mathematical and pharmacological concepts, as well as computer programs. However, although model building is a complex process, models could easily be used to calculate individually modeled kinetic parameters of interest based on a few time points measured during treatment. Of note, these approaches require a higher number of tumor biomarker measurements (generally three to four time points per patient at least) than strategies described above. None of these approaches have been validated prospectively for treatment adjustment in routine or for identification of surrogate biomarkers of treatment efficacy.

discussion

Novel serum biomarkers are emerging as promising prognostic and predictive factors [2–5]. For example, encouraging preliminary results were presented recently on the predictive value of a decline in CTCs in patients with metastatic prostate cancer treated with abiraterone acetate [6, 7]. A single-time point-based approach was used. The best approach for kinetic monitoring has become a relevant issue.

The present review clearly shows the heterogeneity in methodologies used to analyze serum tumor biomarker kinetics over the last 30 years. Despite a large number of publications, few kinetic parameters are currently measured to adjust treatment in routine practice, or used as surrogate biomarkers for clinical studies [2]. The poor utility of reported kinetic parameters may result from the limited value of the biomarkers in their essence, or from inadequate methodologies used to assess them so far. Most reported parameters have relied on simple static approaches based on one or two time points with a limited consideration of intra- and inter-individual variabilities of tumor biomarker assays [2, 90]. Moreover it was frequently unclear if authors identified prognostic or predictive factors of treatment efficacy although these are different concepts [178]. Inconsistencies in the methods and the lack of reproducibility of reported results might have contributed to their progressive abandoning by clinicians.

Longitudinal analysis of tumor biomarker kinetics, offered by modern model-based algorithms, may embody promising alternative strategies despite specific limitations. Integration of multiple measurements necessarily reduces the effects of assay intra- or inter-individual inaccuracies, and enables the calculation of individual mathematical equations for each patient. Relevant information may be retrieved from such analyses in terms of treatment efficacy prediction, or even of cancer diagnosis, as already reported by different international teams [168, 170, 179–182].

For the future, it is imaginable mathematical models might be used to describe tumor biomarker production in relation to tumor size, the effect of treatment doses and dosing schedules, and biomarker elimination. Based on a few time points for each patient, such models might enable the early prediction of disease burden changes or treatment efficacy, which would offer interesting perspectives in terms of early identification of effective doses and dosing schedules of new targeted anticancer agents during drug development [183]. Development of computer programs may facilitate implementation of these models in routine by clinicians who would just have to enter the titers of tumor biomarkers measured serially for their patients.

The kinetic profiles of novel serum tumor biomarkers, such as CTCs or tumor circulating nucleic acids, might provide relevant information regarding treatment efficacy, provided that an appropriate reproducible methodology is used. The pitfalls on the road of the development of traditional serum tumor biomarkers should be avoided for these modern serum biomarkers.
However, the present study suggests that the same heterogeneous methodologies have already been used for assessing them. Inconsistent outcomes have been reported, as illustrated by the range of prognostic baseline cut-offs for CTCs among studies (1 to 5/7.5 ml of blood) [184, 185]. Although the development of a unique method helpful for kinetic analysis of all tumor biomarkers independently on their nature or of their roles is not realistic, efforts for standardization of the approaches may be considered to increase homogeneity of the methods and the comparability of outcomes. They would complement the REMARK recommendations meant to improve reporting of tumor biomarker studies [186]. Such an objective is challenging as it requires an expert consensus regarding the best method, adjustment of this strategy to the different types of biomarker, validation studies and homogenization of analytical kinetic approaches. Ideally, these efforts for should be incorporated into the larger endeavor of the ‘standardization of protocol elements’ to compress NCI-CTEP-supported trial timelines as recommended by the Operational Efficiency Working Group in June, 2010 (http://ctep.cancer.gov/SpotlightOn/OEWG.htm).

disclosure
The authors have declared no conflicts of interest.

Appendix
PubMed search
The following key words were used: ‘kinetics’; ‘prognostic’; ‘predictive’ or terms characterizing the kinetics (‘half-life’; ‘decline’; ‘decrease’; ‘clearance’; ‘percentage decrease’; ‘nadir’, etc.) with ‘tumor biomarkers’ or the names of the principal traditional tumor biomarkers, including antigens (e.g., ‘CA15-3’; ‘CA19-9’, ‘CA125’; α-fetoprotein ‘AFP’; carcinoembryogenic antigen ‘CEA’, etc.); hormones (human chorionic gonadotrophin ‘hCG’); or enzymes (prostate-specific antigen ‘PSA’; lactate dehydrogenase ‘LDH’). Among more than 12,000 references reported by PubMed, 188 original cited articles or recent reviews, considered as the most representative of available methodologies, were retrieved and analyzed. The following information for each approach was collected: biomarker analyzed; kinetic parameters described; methods used to assess the kinetic parameter and the potential prognostic values. The homogeneity in methods and outcomes of similar approaches was analyzed. Moreover, the potential utility of the kinetic parameters, assessed with studied approaches, as surrogate biomarkers of treatment efficacy in research, or as covariates for treatment adjustment in clinical routine as stated by expert consensus, was searched.

methods used to calculate half-lives

One half-life (HL\text{α}) is calculated in the case of mono-exponential curves \{e.g., titer (t) = A \times e^{-\text{α}t} + B\}, whereas two half-lives (HL\text{α} and HL\text{β}) are explored in the case of bi-exponential decline curves \{e.g., titer (t) = A \times e^{-\text{α}t} + B \times e^{-\text{β}t} + C\}.

In some studies, the methodologies were poorly described [113–114]. In other reports, the HL between time-point A and time-point B was calculated using mathematical formula such as [HL = (Ln(2) \times (Time A - Time B))/(Ln(Titer A) - Ln(Titer B))], where Ln is the natural logarithm [105, 115–118]; or [HL = Ln (2)/slope] where the slope was determined graphically [100, 104, 119–120]. When the profile of tumor marker decline was assumed to be mono-exponential, linear regression tests applied at multiple time-points were used to assess decline slopes [96–98, 106, 108, 121–123], whereas nonlinear least-square regression analyses were occasionally used to fit tumor decline profiles according to mono- or bi-exponential equations and then to calculate one or two decline slopes [103, 124–126].

methods used to calculate time to normalization

In some reports, this measure resulted from observations in patients with normalized tumor markers [130–131, 159]. In other studies, time to normalization (TTN) was calculated using a mathematical formula [127], \{TTN (weeks) = 3 \times (log C_0 – log C_t)/(log C_N – log C_t)\}, where C_0 is the baseline tumor marker titer, C_N is the normal upper limit of the tumor marker, and C_t is the tumor marker value after one cycle of chemotherapy. Of note, The TTN-based strategy is not applicable to the treatment of most metastatic cancers in which the normalization of tumor markers is not necessarily expected.

area under the curve

A better integration of the entire decline curve might be achieved by this kinetic parameter when calculated using several time-points. The CA125 area under the curve (AUC) was investigated using the sum of trapezoid areas in localized ovarian cancer patients treated with postoperative chemotherapy [161]. Evidence of the utility of this approach is minimal (or limited) to recommend this approach for the development of new tumor biomarkers.

principles of population kinetic approach

kinetic modeling

Based on a few concentration time-points per patient exposed to a drug, this approach enables, among others, the determination of the kinetics of drug concentrations in the patient population;
quantification of inter- and intra-individual variabilities; and the prediction of the kinetics of drug concentrations in every patient (Figure 3). This strategy presents several advantages, including the ability to work under sparse sampling conditions with data from unselected patients, the determination of individual mathematical equations describing the profiles of tumor biomarker decline independently at selected time-points, the identification of predictive kinetic parameters capable of characterizing the entire decline curve (i.e., clearance, AUC, and residual production).

**references**


