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Topoisomerase II alpha as a marker predicting anthracyclines' activity in early breast cancer patients: Ready for the primetime?

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ABSTRACT

Purpose: This manuscript reviews and discusses results from randomised clinical studies evaluating topoisomerase II alpha (topo II) as a marker predicting anthracyclines' activity in early breast cancer patients.

Methods: A Medline search has led to the identification of six phase III clinical trials, in which topo II has been retrospectively evaluated as a marker predicting anthracyclines' activity in the adjuvant setting.

Results: Rates of topo II gene aberrations, in particular gene deletion, seem to vary substantially between the studies. No extensive correlation has been found between topo II gene status and protein levels. Five of the six trials suggest that topo II gene amplification is associated with increased tumour sensitivity to anthracyclines. Two of the three studies evaluating topo II gene deletions suggest that topo II deleted tumours might also derive an increased benefit from anthracyclines.

Conclusion: Current data suggest that topo II might become a predictive tool to identify patients candidate to receive anthracyclines in the adjuvant setting. Ongoing studies will likely address some pending issues which, at present, prevent the use of this marker in daily practice.

1. Introduction

In the last few years there has been an increasing effort in the attempt to define the role of topoisomerase II alpha (topo II) as a molecular marker predicting the activity of anthracyclines in breast cancer patients.

Topo II protein is the molecular target of topo II inhibitors such as anthracyclines.[1] Topo II protein is a key-enzyme in DNA replication. This enzyme creates a reversible double-strand DNA break allowing the two template strands to rotate freely. This enables the passage of a second DNA double-strand through the break. The topo II gene is located on chromosome 17 q12-q21 next to the HER-2/neu gene.[2] Topo II gene aberrations, either amplifications or deletions, were first reported by a research group from the University of Tampere, Finland.[3] Interestingly, the same group found that topo II aberrations were by far more frequent in HER-2/neu amplified than in HER-2/neu non-amplified tumours.[3] This observation

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is currently explained by the fact that although the HER-2/neu gene is the target gene of amplification at chromosomal region 17 q12-q21, in some cases the amplicon may also harbour other closely related genes including topo II.[3] *In vitro* data from the same group in Finland suggested that breast cancer cells carrying amplification of both genes were highly sensitive to anthracyclines, while HER-2 amplified/topo II non-amplified cells were less sensitive to the same compounds.[4] These pre-clinical data led to formulate the hypothesis that the previously reported correlation between HER-2/neu gene amplification or protein over-expression and response to anthracyclines in breast cancer patients was largely due to concomitant topo II gene aberrations.[5]

2. Phase III clinical studies testing topo II gene aberrations as a predictive marker

The use of fluorescence *in situ* hybridisation (FISH) to detect topo II gene aberrations in fixed samples from breast cancer patients has prompted the activation of six retrospective clinical studies, in which the predictive value of topo II gene aberrations has been evaluated in early breast cancer patients treated with adjuvant chemotherapy in the context of phase III clinical trials (Tables 1a and 1b).[6–12]

The reported studies can be divided into two different subgroups. The first of the two subgroups is characterised by the fact that patients assigned to the study control arm did not receive an anthracycline-based treatment (Table 1a).[6–10] These studies have an ideal design to assess topo II predictive value. In line with the pre-clinical findings,[1–4] all four studies suggest that patients deriving the largest clinical benefit from anthracycline-based chemotherapy have topo II gene amplified tumours.[6–10]

The Belgian study has a modest sample size that makes the study results unstable.[6] The Danish and the Canadian trials evaluated topo II gene status in a larger number of cases ranging between 443 and 773.[7,8] It is important to emphasise that tumours from these two studies were heterogeneous with regard to the HER-2 status.[7,8] This biological heterogeneity might be a confounding factor when the interaction between topo II gene status and response to anthracyclines has to be investigated.

The BCIRG 006 study has the largest sample size and, of note, has evaluated the interaction between topo II gene and anthracyclines in the biologically homogeneous cohort of HER-2 amplified tumours.[9,10] Additional follow-up data from the BCIRG study will be needed because at present the event rate reported in the cohort of topo II amplified tumours is 11.3% (119 events from 1044 patients).[9] Of note, the two so far reported interim analyses from the BCIRG study do suggest that topo II amplified tumours receive the largest benefit from anthracyclines.[9,10] In particular, the two analyses suggest that in the presence of topo II gene amplification, the use of an anthracycline-based therapy could be as effective as two different trastuzumab and chemotherapy-based regimens. Moreover, in topo II non-amplified tumours anthracyclines do not seem to play any relevant role in preventing disease relapse.[9,10] Although the BCIRG study sample size is considerable, the fact that only preliminary follow-up data are available does not allow for definitive conclusions at the present time.

In the second group of studies all patients received an anthracycline-based chemotherapy, although treatments differ for anthracycline's dose-intensity and/or cumulative dose (Table 1b).[11,12] One of two studies suggests that patients deriving the largest benefit from anthracyclines dose-escalation have topo II amplified tumours.[12] Conversely, the other study does not find the expected interaction between anthracyclines and topo II gene status.[11] It is worth mentioning that in this study anthracyclines dose-intensity was below what is nowadays considered the 'standard'.[11] The fact that patients received a somewhat sub-optimal anthracycline-based therapy might explain the lack of interaction between anthracyclines and topo II gene status reported in this study.

Of note, three of the six studies restricted topo II gene testing to the cohort of HER-2/neu amplified tumours,[6,9,12] while the remaining three tested topo II in all patients, independently of the HER-2/neu gene status.[7,8,11] This observation could explain the apparent difference in the rate of topo II gene amplification reported in the studies. When the rate of topo II gene amplification is calculated only among HER-2/neu gene amplified tumours, all the studies report that approximately one-third of cases carry a concomitant topo II gene amplification.

While all the studies have been testing topo II gene amplification as a marker predicting anthracyclines activity, only three of the six studies have tested topo II gene deletions as a predictive marker.[7,8,11] In the remaining three studies,[6,9,12] topo II deleted and topo II normal tumours have been grouped together and defined as topo II non-amplified tumours. Interestingly enough, two of the three studies testing topo II gene deletions suggest that deletions could also identify a patient cohort deriving substantial benefit from anthracyclines[7,8] although pre-clinical data indicate that gene deletion is associated with resistance to these compounds.[4]

3. Issues preventing the use of topo II in daily practice

Although the reported studies suggest that topo II gene amplification defines a cohort of patients deriving the largest benefit from anthracyclines, there are still some pending issues preventing the use of topo II as a valuable predictive tool for daily practice.

3.1. *Topo II gene deletions*

The rate of topo II gene deletions in the six studies exploring topo II as a predictive marker is reported in Tables 1a and 1b. Five of the six studies evaluated topo II gene status by FISH although different probes were used.[6–11] In the remaining one, topo II was tested by chromogenic *in situ* hybridisation (CISH) [12]. Tumours were defined as topo II gene deleted if the ratio between topo II gene copy number and centromere 17 copy number was $\leqslant 0.8$.

Table 1a – Phase III trials in which topo II gene has been tested as a predictive marker

Study	Design	No. topo II evaluable pts	HER-2 status	% topo II gene amplification	% topo gene deletion	Results
Di Leo et al[6] (2002)	CMF HEC EC	61	+	38	13	HEC + EC > CMF if topo II amplified HEC + EC = CMF if topo II non-amplified
Knoop et al[7] (2005)	CMF FEC	773	+/–	12	11	FEC > CMF if topo II amplified or deleted FEC = CMF if topo II normal
O'Malley et al[8] (2006)	CMF FEC	443	+/–	11	6	FEC > CMF if topo II amplified or deleted FEC = CMF if topo II normal
Slamon et al[9] (2006)	AC → DT DPT AC → D	2990	+	35	5	AC → DT = DPT = AC → D if topo II amplified AC → DT = DPT > AC → D if topo II non-amplified

CMF = cyclophophamide-methotrexate-fluorouracil; HEC = standard doses epirubicin-cyclophosphamide; EC = moderate doses epirubicin-cyclophosphamide; AC → DT = doxorubicin-cyclophosphamide → docetaxel-trastuzumab; DPT = docetaxel-carboplatin-trastuzumab; AC → D = doxorubicin-cyclophophamide → docetaxel; FEC = fluorouracil-epirubicin-cyclophophamide.

Table lb – Phase III trials in which topo II gene has been tested as a predictive marker

Study	Design	No. topo II evaluable pts	HER-2 status	% topo II gene amplification	% topo gene deletion	Results
Harris et al[11] (2004)	CAF LD CAF MD CAF SD	624	+/–	7	11	No interaction between CAF and topo II gene status
Tanner et al[13] (2006)	dose-escalated FEC FEC → HD	128	+	37	N.A.	Dose-escalated FEC > FEC if topo II amplified Dose-escalated FEC = FEC if topo II non- amplified

CAF = cyclophosphamide-doxorubicin-fluorouracil; LD = low doses; MD = moderate doses; SD = standard doses; FEC = fluorouracil-epirubicin-cyclophosphamide; HD = high dose non-anthracycline based myeloablative chemotherapy; N.A. = not available.

It can be observed that topo II gene deletion rates range between 4% and 13% (Tables 1a and 1b). If this analysis is carried out only in the group of HER-2 amplified tumours, then the rate of topo II gene deletions ranges between 4% and 24%.

These data suggest that there is some heterogeneity in topo II testing. Of note, results of comparative studies evaluating the performance of the different topo II probes used in the reported studies are lacking. The heterogeneity in topo II testing does not seem to impact substantially on the rate of topo II gene amplification, while it could play a major role in explaining the conflicting rates of topo II gene deletion reported in the different studies.

An additional issue that deserves further consideration is the reported correlation between topo II gene deletions and increased tumour sensitivity to anthracyclines.[7,8] This finding is in contrast with pre-clinical data suggesting that HER-2 amplified/topo II deleted tumours might be resistant to anthracyclines.[4]

A study hypothesis that could explain the correlation between gene deletions and increased sensitivity to anthracyclines is that deletions might involve one of the two alleles of the topo II gene and that the wild-type allele might compensate in the presence of conditions requiring a high level of topo II activity. This compensation mechanism could happen for a gene such as topo II and its promoter that seem to be controlled at different levels including the transcription and the translation phases[13–16] (Fig. 1). At present, the lack of studies measuring topo II protein levels and/or protein activity in topo II deleted tumours makes it impossible to confirm this hypothesis.

3.2. *Lack of extensive correlation between topo II gene status and protein levels*

Four studies have correlated topo II gene status and protein levels in primary breast cancer samples.[17–20] Three of the four studies are suggesting the lack of extensive correlation between topo II gene status and topo II protein levels measured by immunohistochemistry (Table 2).[17,18,20]

This finding is supported by the fact that, unlike the expression of HER-2/neu, topo II protein expression is highly regulated at the level of transcription and translation in a way that gene amplification may not dramatically alter protein levels.[13] Interestingly enough, the topo II gene promoter responds to the increased Ras activity associated with cell proliferation[14] and transcription is coordinated according to the cell cycle phase.[15] At the m-RNA level, topo II is regulated through untranslated 3′ sequences that control topo II m-RNA stabilisation.[16]

To reinforce the concept that topo II protein levels are governed by cell proliferation, several studies have reported in the past a significant degree of correlation between topo II protein levels and proliferation markers.[21–23] Last but not the least, one of the four studies correlating gene status with protein levels divided the study population into two cohorts according to Ki-67 scores.[17]

Of note, in the cohort of highly proliferating tumours (i.e. Ki-67 > 25%) topo II gene amplification was significantly correlated with protein over-expression, while the same correlation was lacking in the remaining cohort of slowly proliferating tumours.[17]

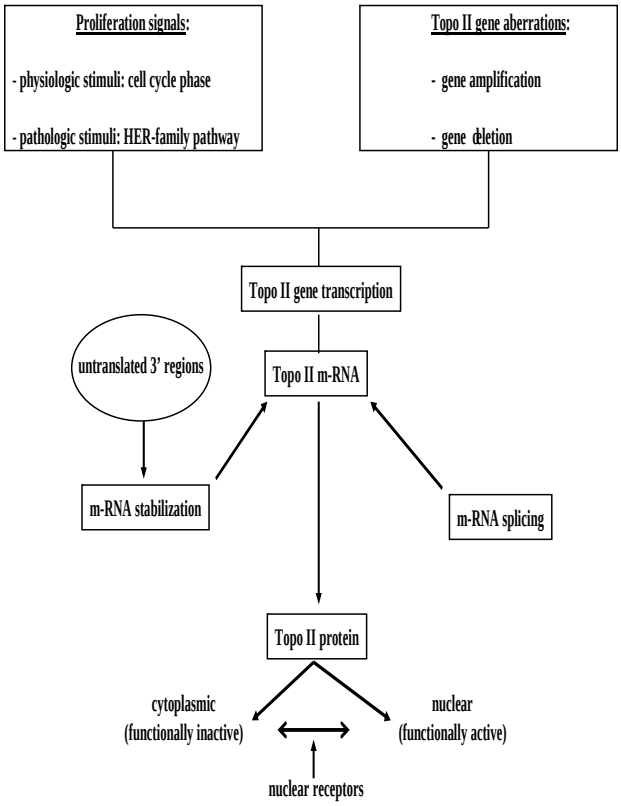

Fig. 1 – Factors involved in the control of topo II protein levels and enzymatic activity. The level of topo II gene transcription is controlled by proliferation signals and by gene status.[2–4,13–15,21–23] Fast-growing tumours carrying topo II gene amplification are expected to have high levels of topo II gene transcription.[17] A second control mechanism plays at the m-RNA level. Untranslated 3′ regions govern topo II m-RNA stabilisation and ultimately protein levels,[16] while m-RNA splicing lead to different topo II isoforms.[27] Truncated isoforms are not transported to the nucleus and remain localised in the cytosol. Full-length isoforms are transported from the cytosol to the nucleus and are functionally active.[28–31] Nuclear receptors, under conditions such as proliferation signals, could regulate the transportation of isoforms from the cytosol to the nucleus and *vice versa*.[30,31]

These data indicate that tumour proliferation regulates topo II protein levels and that both gene amplification and tumour proliferation status play a key-role in controlling protein levels.

An additional factor that could explain the lack of an extensive correlation between gene and protein status is the sub-optimal performance of immunohistochemistry in measuring topo II protein levels in routinely fixed tumour samples. Immunohistochemistry allows for the estimation of the tumour cells fraction carrying topo II immunostaining. This fraction almost corresponds to the tumour cells proliferation rate because topo II is a key-enzyme involved in DNA replication.[13]

Table 2 – Studies correlating topo II gene status with protein levels in primary breast cancer

Study (year)	No. of evaluable cases	HER-2 status	Type of topo II testing		Results	
			Gene	Protein	Gene status	Protein status
Durbecq et al.[17]	103	+	FISH	IHC	A (n = 38) NA (n = 65)	17%[a] 12%[a]
Mueller et al.[18]	81	+/–	FISH	IHC	A (n = 14) NA (n = 67)	22%[a] 19%[a]
Bhargava et al.[19]	104	+/–	CISH	IHC	A (n = 25) NA (n = 79)	72%[b] 4%[b]
Callagy et al.[20]	178	+/–	FISH	IHC	A (n = 28) NA (n = 150)	93%[b] 71%[b]

FISH = fluorescence *in situ* hybridization; CISH = chromogenic *in situ* hybridization; IHC = immunohistochemistry; A = amplified; NA = non-amplified; n = number of patients.
a Expressed as mean % of immunostained cells.
b Expressed as % of cases with protein overexpression.

3.3. *Predictive role of topo II protein levels independently of topo II gene status*

The predictive value of topo II protein levels has been investigated independently of gene status in two retrospective studies in which breast cancer patients were randomly treated with either an anthracycline-based or with another cytotoxic regimen for early[24] or advanced[25] breast cancer.

In the first of the two studies 481 archival primary tumour samples from breast cancer patients were centrally evaluated for topo II protein levels by immunohistochemistry.[24] Patients were participating in a phase III clinical trial comparing an anthracycline-based treatment (EC: epirubicin-cyclophosphamide) to classic CMF as adjuvant therapies for node-positive disease. Topo II protein over-expression was defined as the detection of nuclear immunostaining in more than 10% of evaluated cells. Thirty-four percent of cases were classified as topo II positive (i.e. presence of over-expression). With a median follow-up of 50 months, event-free survival (EFS) was compared between patients treated with EC or CMF in the two cohorts of topo II positive or negative tumours. Interestingly, in the cohort of topo II positive tumours better EFS was reported for patients treated with EC than with CMF. The EFS benefit was not observed in the remaining cohort of topo II negative patients (Topo II positive cohort: hazard ratio 0.66, 95% confidence intervals (CI) 0.32–1.36, $p = 0.25$; Topo II negative cohort: hazard ratio 1.26, 95% confidence intervals 0.63–2.50, $p = 0.51$; interaction test p value: 0.13). Of note, the same clinical trial compared two EC regimens where epirubicin and cyclophosphamide dose-intensities and cumulative doses were different. The most intensive EC showed superiority over the less intensive regimen only in the cohort of patients carrying topo II over-expression.[24] Recently, the National Cancer Institute – Canada Group has presented the results of a very similar retrospective phase III clinical trial confirming that topo II protein over-expression might be associated with increased benefit from anthracyclines over CMF in the adjuvant setting.[26]

In the second study, 108 archival primary tumour samples from breast cancer patients were centrally evaluated for topo II protein levels by immunohistochemistry.[25] Patients participated in a phase III clinical trial comparing full dose single-agent doxorubicin to full dose single-agent docetaxel as first/second-line treatment for advanced disease. No correlation between topo II protein levels and probability of objective response to docetaxel was found, while a direct correlation was observed between protein levels and doxorubicin clinical activity. When topo II protein levels were treated as a continuous variable, a 10% increase in the percentage of immunostained cells was associated to a 9% increase in the probability of response to doxorubicin (hazard ratio 1.09, 95% confidence intervals 1.03–1.15, $p = 0.002$). Conversely, no increase in the probability of response to docetaxel according to topo II protein levels was reported (hazard ratio 1.002, 95% confidence intervals 0.94–1.07, $p = 0.95$).[25]

It is important to emphasise that these studies evaluated topo II protein levels as a predictive marker independently of topo II gene status. The studies suggest that protein levels could define a cohort of patients deriving the largest benefit from anthracyclines.

4. A multi-factorial model

Available data reviewed in the present manuscript suggest that topo II gene amplification as well as topo II protein over-expression might predict one independently of the other, clinical response to anthracyclines.[6–12,24–26] Interestingly enough, pre-clinical and clinical data support the concept that tumour proliferation status can play a key-role in regulating topo II protein levels, independently of topo II gene status. Topo II gene and proliferation status seem to be equally important in governing protein levels.[13–23] Moreover, post-translational mechanisms involved in the regulation of topo II m-RNA stabilisation could also be involved in the control of protein levels.[16]

An additional variable that could impact on the level of activity of topo II protein is its subcellular distribution. It has been reported that different topo II isoforms are expressed through alternative splicing and that some of these isoforms have a prevalent cytoplasmic localisation.[27] *In vitro*, topo II cytoplasmic localisation seems to be associated with resistance to topo II inhibitors.[28,29] It has been described that topo II can be transported between the nucleus and cytoplasm. The majority of data regard the description of topo II

import in the nucleus from the cytosol. More recently, a cellular mechanism leading to the topo II nuclear export has been described. Interestingly, this process seems to be mediated by nuclear export signals involving some nuclear receptors such as CRM-1.[30,31] These data seem to support the concept that topo II catalytic activity does not necessarily correlate with cellular protein levels and that additional mechanisms such as protein localisation could play a role in controlling protein activity in each individual tumour (Fig. 1).

To corroborate the evidence of a multi-factorial model, data on topo II gene status in advanced breast cancer patients experiencing a complete response to anthracycline-based therapy are available.[32] In this study, of the 14 complete responders only 3 had topo II gene amplification in the primary tumour. Interestingly, in 1 of the 17 patients reporting disease progression as the best response to an anthracycline-based chemotherapy, the primary tumour carried topo II gene amplification.[32]

These data support the concept that the prediction of response to anthracyclines seems to depend on more than one single variable. The ongoing TOP trial, coordinated in Brussels at the Jules Bordet Institute, might provide us with new insights. In the TOP trial early breast cancer patients with endocrine-resistant disease are treated pre-operatively with single-agent full-dose epirubicin. The study primary end-point is the correlation between pathological complete remission and molecular markers evaluated on a pre-epirubicin sample. Topo II gene and protein, proliferation markers and genomic profiles are investigated. This prospectively designed trial might confirm in a clinical setting the complex topo II regulation network illustrated in Fig. 1, and might even disclose other molecular markers potentially involved in the prediction of response to anthracyclines.

5. Conclusions

Although pre-clinical and clinical data discussed in the present manuscript suggest that topo II gene and protein testing might provide the clinician with potentially valuable information for clinical practice, we are not yet ready for the use of topo II as a standard predictive tool for those patients candidate to receive an anthracycline-based therapy.

Critical issues that need to be addressed are a full understanding of topo II regulation mechanisms, the role of cellular events such as proliferation in controlling topo II activity, a better standardisation of topo II gene testing and a more accurate evaluation of topo II protein level of activity.

Last but not the least, additional evidence from the previously reported clinical trials that have compared anthracyclines with a non-anthracycline-based therapy would be highly important to corroborate the early findings. Topo II testing in these trials would be helpful in the attempt to validate the results obtained from the available clinical studies.

A current project attempts to validate topo II as a predictive marker in a population of almost four thousands early breast cancer patients treated in the adjuvant setting with either anthracyclines or CMF in the context of four different clinical trials.[33–36] In this study, a meta-analysis of clinical data from the four trials and a centralised evaluation of HER-2/neu and topo II genes by FISH on primary tumour samples is ongoing under the coordination of our institution in Prato and with the collaboration of the Jules Bordet Institute, Brussels, the Danish Breast Cancer Cooperative Group, the National Cancer Institute of Canada and the NEAT (English-Scotland) cooperative group. The statistical analysis is carried out by the International Drug Development Institute in Brussels, while HER-2/neu and topo II testing is ongoing at the Institute of Medical Technology, University of Tampere, Finland.

In conclusion, further biological and clinical evidence are needed to consider topo II as a valuable predictive marker for daily practice. Ongoing studies will provide us with additional data that will contribute to define topo II value in breast cancer patients candidate to receive anthracycline-based therapies.

Conflicts of interest Statement

A. Di Leo has received honoraria from Abbott Molecular and from Dako Denmark A/S for participation in advisory board meeting and as a speaker at satellite symposia.

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