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Topoisomerase expression and amplification in solid tumours: Analysis of 24,262 patients

Gregory M. Heestand[a,1], **Maria Schwaederle**[a,*], **Zoran Gatalica**[b], **David Arguello**[b], and **Razelle Kurzrock**[a]

[a]Center for Personalized Cancer Therapy, UC San Diego Moores Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093, USA

[b]Caris Life Sciences, 4750 South 44th Place, Phoenix, AZ 85040, USA

Abstract

Background—Topoisomerase I (TOPO1) and topoisomerase IIα (TOP2A) are specific targets of multiple chemotherapy drugs. Increased expression of TOPO1 protein and amplification of the *TOP2A* gene have been associated with treatment response in colorectal and breast cancers, respectively. TOPO1 and TOP2A may be potential therapeutic targets in other malignancies as well.

Summary of methods—We analysed TOPO1 protein expression and *TOP2A* gene amplification in patients (n = 24,262 specimens) with diverse cancers. Since *HER2* and *TOP2A* co-amplification have been investigated for predictive value regarding anthracycline benefit, we analysed specimens for *HER2* amplification as well.

Results—Overexpressed TOPO1 protein was present in 51% of the tumours. Four percent of the tumours had *TOP2A* amplification, with gallbladder tumours and gastroesophageal/oesophageal tumours having rates over 10%. Overall, 4903 specimens were assessed for both *TOP2A* and *HER2* amplification; 129 (2.6%) had co-amplification. High rates (>40%) of *HER2* amplification were seen in patients with *TOP2A* amplification in breast, ovarian, gastroesophageal/oesophageal and pancreatic cancer.

Conclusion—Our data indicate that increased TOPO1 expression and *TOP2A* amplification, as well as *HER2* co-alterations, are present in multiple malignancies. The implications of these observations regarding sensitivity to chemotherapy not traditionally administered to these tumour types merits investigation.

*Corresponding author: University of California, San Diego Moores Cancer Center, Center for Personalized Cancer Therapy, 3855 Health Sciences Drive, #0658, La Jolla, CA 92093, USA. Fax: +1 (858) 822 2300.

[1]Present Address: Division of Oncology, Stanford University School of Medicine, 875 Blake Wilbur Drive, Stanford, CA 94305, USA.

Conflict of interest statement

Zoran Gatalica and David Arguello are employees of Caris Life Sciences. Razelle Kurzrock has research funding from Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine and Guardant Health, as well as consultant fees from Sequenom and Actuate Therapeutics and an ownership interest in Novena, Inc. and Curematch, Inc. The other authors have no conflict of interest to disclose.

Keywords

Topoisomerase I; Topoisomerase IIα; HER2; Genomic profiling; Solid tumors

1. Introduction

The topoisomerase family of enzymes plays a key role in unwinding coiled DNA to facilitate replication and transcription. By reversibly cleaving the DNA backbone, topoisomerase allows tension in the double helix to be released. There are two types of topoisomerase enzymes: type I enzymes cleave one of the two backbones in double-stranded DNA allowing the double helix to untwist, whereas type II enzymes cleave both DNA backbones allowing for a strand of supercoiled DNA to pass through the break before reconnecting [1,2].

Because topoisomerase enzymes regulate DNA function, they are potential targets for cancer treatment. Multiple classes of chemotherapy drugs have been developed accordingly. Camptothecin directly inhibits the activity of topoisomerase I (TOPO1) [3]. Two derivatives of camptothecin, irinotecan and topotecan are in broad clinical use. Etoposide and the anthracycline chemotherapies doxorubicin, daunorubicin, and epirubicin inhibit topoisomerase IIα (TOP2A) by blocking its ability to repair DNA strands after being cleaved [4]. The net impact is to interrupt reproduction of cancerous cells.

Tumour samples can be assayed in the laboratory for TOPO1 and TOP2A protein expression by immunohistochemistry (IHC). Although the TOPO1 IHC assay has been used in multiple clinical studies [5–7], the clinical relevance of TOP2A protein expression is less clear. TOP2A protein expression does not necessarily correlate with the amplification of its encoding gene, *TOP2A*, though *TOP2A* amplification has been associated with benefit from anthracycline chemotherapy [8,9].

HER2, which encodes the HER2 tyrosine kinase critical to cell signalling and shares the long arm of chromosome 17 with *TOP2A*, has also been implicated in anthracycline sensitivity [10]. For patients with *HER2* amplification receiving anthracyclines, co-amplification of *TOP2A* has been associated with improved outcomes [11–14]. HER2 can be targeted clinically by several drugs including the monoclonal antibody trastuzumab, which improves survival when added to conventional chemotherapy in patients with *HER2* overexpression [15,16].

In this article, we studied TOPO1 expression in 23,586 tumour samples and *TOP2A* amplification in 5171 tumour samples (total = 24,262 patients) with the goal of identifying cancer types that may respond to topoisomerase inhibitors. Because of the importance of *HER2* in patients with *TOP2A* amplification, as noted above, we also studied the co-amplification of *TOP2A* and *HER2* in 4903 specimens assayed for both the genes.

2. Materials and methods

2.1. Tissue samples

Test results of consecutive tissue samples (January 2012–August 2014) of locally advanced and/or metastatic solid tumours submitted to a commercial clinical laboratory improvements amendments molecular profiling laboratory (Caris Life Sciences, Phoenix, AZ) were reviewed. (Samples are interrogated based on ordering physician request). Multiplatform profiling included IHC and fluorescence *in-situ* hybridisation (FISH). Since the study included only deidentified data, it was considered exempt by the UC San Diego Internal Review Board.

2.2. TOPO1 immunohistochemistry

IHC analysis was performed on formalin-fixed paraffin-embedded tumour samples using commercially available antibodies against TOPO1 (1D6, Leica Biosystems, Germany). All slides were read by a board-certified pathologist. Slides were scored as 0+, 1+, 2+, or 3+ depending on the staining intensity, and percent tumour stained was also assigned. The predetermined threshold to determine TOPO1 overexpression was a staining intensity of 2+ or 3+ in at least 30% or more tumour cells on a given slide. All IHC assays were performed using commercially available detection kits and automated staining techniques (Benchmark XT, Ventana Medical Systems, Tucson, AZ and AutostainerLink 48, Dako, Denmark).

2.3. TOP2A fluorescent in-situ hybridisation

FISH was used for evaluation of *TOP2A* using a commercial probe for *TOP2A* and the pericentromeric region of chromosome 17 (Vysis *TOP2/CEP17* probe, Abbott Molecular, Des Plaines, IL). *TOP2A* was determined in a minimum of 20 inter-phase tumour cell nuclei and compared with chromosome 17 centromeres in those tumour nuclei. *TOP2A* amplification was defined as a *TOP2A/CEP17* signal ratio ≥2.0.

2.4. HER2 in-situ hybridisation (ISH)

FISH and chromogenic *in-situ* hybridisation (CISH) were used interchangeably to evaluate *HER2* amplification.

FISH was performed with a probe specific for *HER2* (17q11.2-q12 region) and a probe for the pericentromeric region of chromosome 17 (Pathvysion, Abbott Molecular). Inter-phase nuclei were examined and the ratio of *HER2* signals to chromosome 17 centromere signals were evaluated to indicate amplification status of this gene. A *HER2/CEP17* ratio higher than 2.2 was considered amplified (ISH+); a *HER2/CEP17* ratio between 1.8 and 2.2 (equivocal) and a *HER2/CEP17* ratio <1.8 (negative) were both considered non-amplified (ISH−). The Pathvysion *HER2* probe has been approved by the US Food and Drug Administration for selection of patients for trastuzumab and pertuzumab therapy.

CISH was performed by using the INFORM *HER2* Dual ISH DNA Probe Cocktail (Ventana Medical Systems) to determine *HER2* gene status by enumeration of the ratio of the *HER2* gene to chromosome 17. The *HER2* and chromosome 17 probes were detected using two-colour *in-situ* hybridisation in formalin-fixed, paraffin-embedded human cancer tissue

specimens following staining on the BenchMark XT automated slide stainer and visualised by light microscopy. A *HER2/CEP17* ratio higher than 2.0 was considered amplified (ISH+), whereas a *HER2/CEP17* ratio <2.0 was considered non-amplified (ISH−). The INFORM *HER2* Dual ISH DNA Probe Cocktail has been approved by the US Food and Drug Administration for selection of patients to *HER2*-targeted therapies in breast cancer.

2.5. Statistical methods

Descriptive statistics was used for most analyses. JMPv11.1.1 (SAS Institute Inc., Cary, NC) was utilised for statistical analysis.

3. Results

3.1. TOPO1 expression

Tumour samples from 23,586 patients were stained for TOPO1 expression using IHC (Supplemental Table 1). Fifty-seven cancer subtypes were represented. TOPO1 was overexpressed in 51% of the tumours. TOPO1 over-expression was also present in >60% of the patients with each of small cell lung, gastroesophageal and oesophageal, thymic, gastric, anal, breast, prostate and poorly differentiated neuroendocrine cancers (Table 1, includes only tumours with at least 40 specimens). TOPO1 was over-expressed in 47% of the colon tumours. There were also several other tumour types in which a majority of patients expressed high levels of TOPO1, but less than 40 samples were assayed (Supplemental Table 1).

3.2. TOP2A amplification

Tumour samples from 5171 patients were assayed for *TOP2A* amplification using FISH (Supplemental Table 1). Fifty-one cancer subtypes were represented. *TOP2A* amplification was present in 4.0% of the tumours. Most notably, *TOP2A* amplification was present in 17% of gallbladder cancers and in 12% of gastroesophageal and oesophageal cancers (Table 2). *TOP2A* amplification was also present in 5.0% of invasive breast cancers.

3.3. HER2 amplification and co-amplification with topoisomerase

HER2 amplification data were analysed on 10 tumour types with the highest *TOP2A* amplification (Fig. 1). Overall, 4903 patients were analysed for both *TOP2A* and *HER2* and 129 (2.6%) had co-amplification. Of 202 patients with *TOP2A* amplification who were analysed for *HER2*, 129 (64%) had *HER2* amplification; of 483 patients with *HER2* amplification who were analysed for *TOP2A* amplification, 129 (27%) had *TOP2A* amplification (Fig. 2).

Twenty-three percent of gallbladder cancers (5 of 22 patients, all tested for *TOP2A and HER2)* had *HER2* amplification, with co-amplification of both *HER2* and *TOP2A* in 18% (n = 4). Fifteen percent of gastro-esophageal and oesophageal cancers (10 of 65 patients, all tested for *TOP2A a*nd *HER2*) had *HER2* amplification, with co-amplification of both *HER2* and *TOP2A* in 7.7% (n = 5 patients). Sixty-three percent of gastroesophageal and esophageal tumours with *TOP2A* amplification also had *HER2* amplification (5 of 8

patients), whereas 50% with *HER2* amplification also had *TOP2A* amplification (5 of 10 patients).

4. Discussion

Topoisomerase enzymes are expressed in multiple tumour types and are potential targets for cancer treatment. To date, the most relevant to cancer care are TOPO1 and TOP2A. TOPO1 has been extensively studied in colorectal cancer—two large retrospective studies have suggested that high levels of TOPO1 are associated with increased survival when patients are treated with combination chemotherapy [5], one of which specifically associated this benefit with irinotecan-based chemotherapy [6]. Braun *et al.* [5] screened 1628 patients from the FOCUS trial for predictive bio-markers using archived tissue. Patients enrolled in this trial had newly diagnosed metastatic colorectal cancer and were treated with either sequential or combination chemotherapy regimens containing fluorouracil, oxaliplatin, or irinotecan. Of the enrolled patients, 1313 were assessable for TOPO1 protein expression. Patients with high TOPO1 expression (>50% nuclear staining) had a median survival improvement of 5.3 months ($p = 0.005$) when treated with combination chemotherapy upfront compared with sequential fluorouracil. There was no benefit in patients with moderate or low TOPO1 expression. Similarly, Kostopolous *et al.* [6] studied 498 patients who received adjuvant therapy for resected colon cancer and quantified TOPO1 protein expression from archived tumour specimens. In multivariate analysis including treatment with irinotecan, patients with high TOPO1 expression lived longer (HR = 0.61, 95% CI 0.42–0.88, $p = 0.009$). Of the elevated TOPO1 subgroup, patients treated with an irinotecan-containing regimen had improved survival (HR = 0.47, 95% CI 0.23–0.94, $p = 0.033$). The issue remains controversial, however, as other colorectal studies have not identified a survival correlation between irinotecan-containing therapy and TOPO1 expression [17].

TOP2A amplification has been similarly implicated as a biomarker for anthracycline sensitivity in breast cancer. In the Danish Breast Cancer Cooperative Group trial 89D, 980 patients with resected breast cancer were randomised to nine cycles of chemotherapy with cyclophosphamide, epirubicin, fluorouracil (CEF) versus cyclophosphamide, methotrexate, fluorouracil (CMF). Patients with *TOP2A* amplification who received the anthracycline epirubicin in the CEF arm had improved relapse-free survival compared with patients with *TOP2A* amplification receiving CMF (HR 0.43, 95% CI 0.24–0.78, $p = 0.01$) [18]. Amplification and deletion of *TOP2A* have both been implicated as predictive of response to anthracyclines in retrospective studies and meta-analyses [19–21]. Though TOP2A protein (the product of the *TOP2A* gene) can be assayed by IHC, FISH for *TOP2A* is the preferred diagnostic modality. In a 149 patient neoadjuvant study using single-agent epirubicin, *TOP2A* amplification by FISH was associated with pathological complete response ($p \leq 0.001$), but not TOP2A protein expression by IHC ($p \leq 0.33$) [9]. For patients with *HER2* amplification receiving anthracyclines, co-amplification of *TOP2A* has been associated with improved outcomes [11–14]. These findings must be interpreted with caution, however, as not all studies have demonstrated a correlation between *TOP2A* amplification and anthracycline sensitivity [22], and at least one suggests that TOP2A gene expression may be a better biomarker than amplification [23].

As colon and breast cancers are relatively common, topoisomerase expression and its predictive value in these cancers has been extensively studied. However, the role of topoisomerase expression in other malignancies is less well known.

We found that TOPO1 expression and *TOP2A* amplification are present in a large number of tumour types beyond colorectal and breast cancers. Most notably, 73% of small cell lung cancers and 62% of poorly differentiated neuroendocrine cancers overexpress TOPO1 (Table 1). These tumour types can be histologically similar and both are traditionally treated with cisplatin/etoposide combination chemotherapy. Based on the high percentage of patients with TOPO1 over-expression, treatment with irinotecan would be worth investigating. Indeed, several studies have evaluated irinotecan in small cell lung cancer, both as a single-agent and as part of a platinum doublet. As a single-agent, irinotecan has a reported response rate of 47% in patients with relapsed or refractory disease [24]. A phase III study randomising 154 newly diagnosed extensive-stage patients with small cell lung cancer to cisplatin/irinotecan versus cisplatin/etoposide was stopped early due to a median survival improvement of 12.8 versus 9.4 months favouring the irinotecan arm ($p = 0.002$) [25].

In patients with gastroesophageal and oesophageal cancers, the incidence of TOPO1 overexpression is 66% and *TOP2A* amplification is 12%. Fluorouracil/irinotecan is already routinely used as a first-line regimen in patients with advanced disease, with a reported median survival of 9.0 months [26]. For fit patients with advanced disease, the combination of epirubicin, oxaliplatin, and capecitabine offers a median survival of 11.2 months [27].

TOP2A was amplified in 17% of the patients with gallbladder cancer, suggesting that there may be a role for anthracycline chemotherapy or etoposide in this disease. The current standard of care for advanced gallbladder cancer is gemcitabine/cisplatin with a response rate of 38% [28], and there are data supporting the use of regimens-containing fluorouracil and oxaliplatin as well [29]. Two small studies added epirubicin to cisplatin/fluorouracil and cisplatin/capecitabine backbones and reported response rates of 19% and 40% respectively in patients with advanced biliary cancers including gallbladder cancer [30,31]. Epirubicin, oxaliplatin and capecitabine combination may be a reasonable alternative to study in patients with *TOP2A* amplification. Considering that all gallbladder cancer patients with *TOP2A* amplification reported in this study also have *HER2* co-amplification (albeit with only a small number of patients positive for *TOP2A* amplification that were tested for *HER2* amplification; $n = 4$), it would be tempting to add trastuzumab to this regimen as well. However, combining trastuzumab and an anthracycline is not routinely recommended due to the risk of cardiotoxicity.

Sixty-four percent of patients with *TOP2A* amplification also had *HER2* co-amplification (129 of 202 patients). This may be due to the location of both genes on chromosome 17, though only 27% of the patients with *HER2* amplification also had *TOP2A* co-amplification (129 of 483 patients). As HER2 can be targeted with trastuzumab, it may be reasonable to test patients with *TOP2A* amplification reflexively for *HER2* amplification to identify additional treatment options [32]. Of course, the number of patients with co-amplification is small, and larger subsets would be needed to confirm the frequency of the co-amplification phenomenon.

There are several limitations to this study. While the overall number of patients is very large, in some cancers, there were small or variable numbers of patients. In the TOPO1 IHC data set, the number of patient samples per tumour type ranged from five samples to 4703 samples. In the *TOP2A* FISH data set, the number of samples per tumour type ranged from one sample to 2540 samples. Only *TOP2A* amplification was characterised, not *TOP2A* deletion, and *TOP2A* amplification results could be affected if *HER2* overlapped on the same amplicon. Six tumour types included in the TOPO1 IHC data set did not have specimens available for the *TOP2A* FISH assay. For tables included in this article, we displayed only tumour types with at least 40 (Table 1) or 20 patients (Table 2 and Fig. 1). Due to a lack of a standard methodology and threshold, discrepant results were found between our results and other publications. Further studies including annotated data for clinical correlations could not be performed because pertinent clinicopathologic information was unavailable.

In summary, increased TOPO1 expression and *TOP2A* amplification are present in multiple malignancies. Although chemotherapeutic agents are often distinguished from “targeted” agents and are generally given to patients without biomarker selection, it is plausible that TOPO1 and *TOP2A* should be further investigated for their capacity to predict response. It is also reasonable to ask if the presence of these high expression or amplification levels correlate with sensitivity to chemotherapy not traditionally associated with specific tumour types. Further investigation is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejca.2017.06.019.

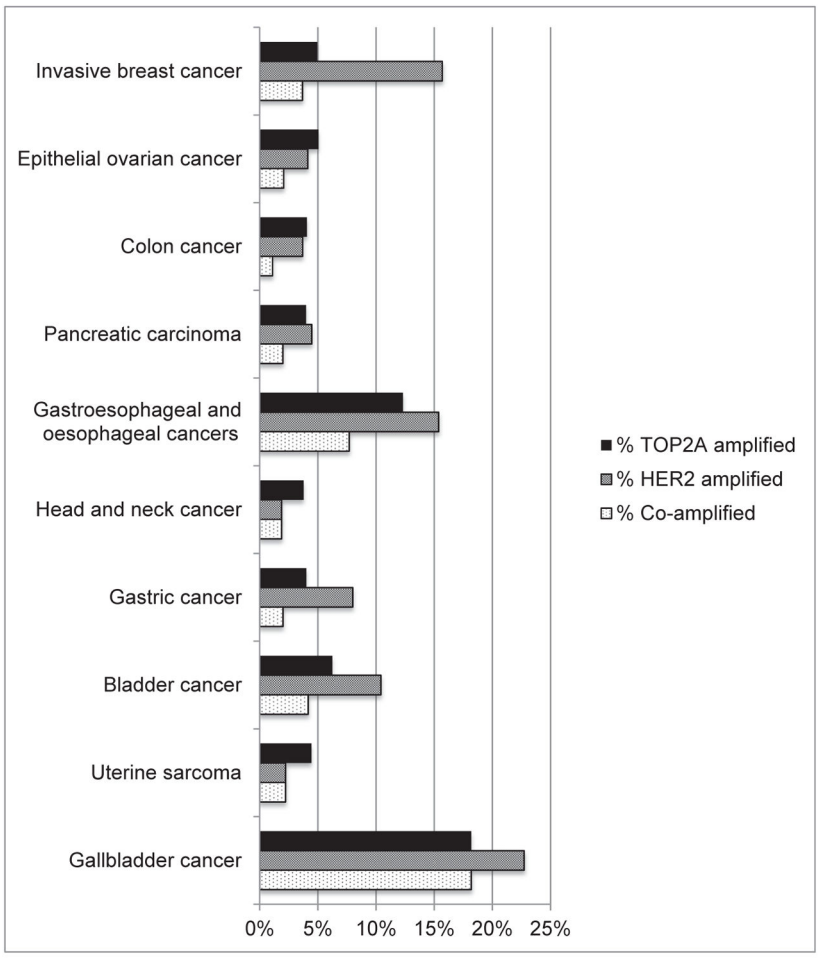

Fig. 1.

TOP2A and *HER2* amplification by FISH/CISH*.

*Only malignancies with at least 20 patient samples are reported. Only patients who were tested for both *TOP2A* and *HER2* amplification were included.

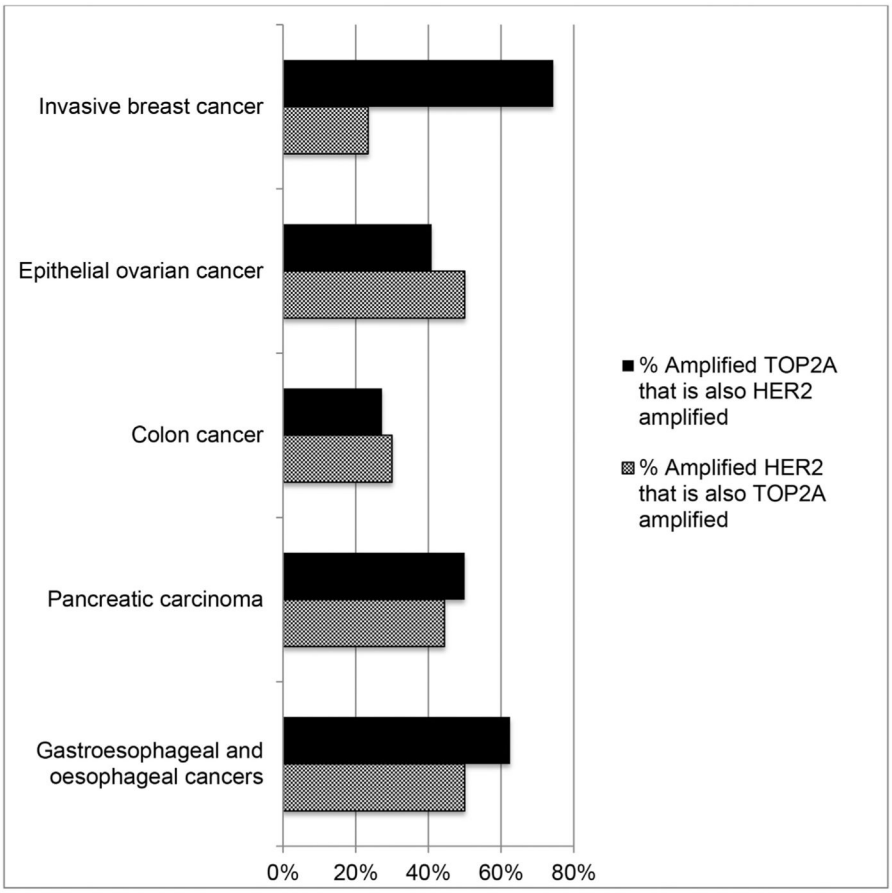

Fig. 2.

Percent *TOP2A* and percent *HER2* co-amplified by FISH/CISH*.

*Only malignancies with at least 5 patient samples that exhibit *TOP2A* amplification are reported.

Table 1

TOPO1 overexpression by IHC in 23,586 patients with diverse malignancies.[a]

	Overexpressed	Total	Percent
Small cell lung cancer	143	195	73.3%
Gastroesophageal and oesophageal cancers	266	401	66.3%
Thymoma and thymic cancer	35	54	64.8%
Gastric cancer	208	322	64.6%
Anal carcinoma	68	106	64.2%
Invasive breast cancer	1976	3119	63.4%
Prostate cancer	141	226	62.4%
Poorly differentiated neuroendocrine tumour	116	187	62.0%
Malignant pleural mesothelioma	50	84	59.5%
Occult primary	447	752	59.4%
Extrahepatic cholangiocarcinoma	26	45	57.8%
Cervical cancer	221	385	57.4%
Osteosarcoma and dedifferentiated chondrosarcoma	32	56	57.1%
Rectal cancer	187	331	56.5%
Intrahepatic cholangiocarcinoma	123	220	55.9%
Bladder cancer	164	294	55.8%
Anaplastic gliomas and glioblastoma multiforme	278	514	54.1%
Ovarian sex-cord and stromal tumours	79	150	52.7%
Non-small cell lung cancer	1360	2587	52.6%
Pancreatic carcinoma	600	1155	51.9%
Bladder cancer: upper genitourinary tract	48	94	51.1%
Head and neck cancer	173	339	51.0%
Adult low-grade infiltrative astrocytoma and oligodendroglioma	31	61	50.8%
Gallbladder cancer	60	120	50.0%
Basal cell and squamous cell cancer	37	78	47.4%
Colon cancer	1067	2258	47.3%

[a]The 26 malignancies with the highest percentage of overexpression are represented. Overexpression is defined as 2+ by IHC and only malignancies with at least 40 patient samples are reported. See Supplemental Table 1 for full list.

Table 2

TOP2A amplification by FISH in 5171 patients with diverse malignancies.[a]

Malignancy type	Amplified	Total	Percent
Gallbladder cancer	4	23	17.4%
Gastroesophageal and oesophageal cancers	8	68	11.8%
Bladder cancer	3	49	6.1%
Invasive breast cancer	126	2540	5.0%
Epithelial ovarian cancer	23	510	4.5%
Uterine sarcoma	2	46	4.3%
Gastric cancer	2	50	4.0%
Colon cancer	11	277	4.0%
Pancreatic carcinoma	8	209	3.8%
Head and neck cancer	2	55	3.6%
Non-small cell lung cancer	9	314	2.9%
Rectal cancer	1	37	2.7%
Occult primary	2	101	2.0%
Endometrial carcinoma	4	232	1.7%
Cervical cancer	1	60	1.7%
Anaplastic gliomas and glioblastoma multiforme	1	79	1.3%
Melanoma	0	75	0.0%
Carcinoid tumour	0	48	0.0%
Poorly differentiated neuroendocrine tumour	0	37	0.0%
Prostate cancer	0	36	0.0%

[a]The 20 malignancies with the highest percentage of *TOP2A* amplification are represented. Only malignancies with at least 20 patient samples are reported. See Supplemental Table 1 for full list.