

Original Article

Comparative immunohistochemical expression of TROP2 in brain metastatic and primary tumors

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Abstract: TROP2 overexpression was reported to predict poor prognosis and increased metastatic potential. This study evaluates TROP2 expression in brain metastases from diverse solid tumors and its consistency with primary tumors, addressing the limited research on TROP2 heterogeneity and its implications for CNS-targeted therapies. TROP2 immunohistochemical staining was performed on 61 brain metastatic tumors and 14 corresponding primary tumors. Based on TROP2 expression, cases were categorized into three groups: diffuse positive (> 95%), focal positive (0-95%), and negative (0%). Among the 61 brain metastatic tumors, TROP2 expression was diffusely positive in 62.3%, focally positive in 16.4%, and negative in 21.3%. Diffuse TROP2 positivity was most observed in tumors of lung origin, while focal positivity was predominant in colorectal metastases. In terms of pathologic diagnosis, adenocarcinoma was the most common type, with 55.6% showing diffuse positivity, 33.3% showing focal positivity, and 11.1% showing negativity. Notably, all cases of invasive breast carcinoma of no special type (IBC-NST) exhibited diffuse TROP2 positivity. No significant association was found between TROP2 expression levels and post-CNS metastasis survival. In the paired analysis of 14 cases with both primary and metastatic tumors, TROP2 expression was consistent between primary and metastatic sites in 78.6% of cases. However, 21.4% of cases, including colorectal adenocarcinoma and kidney clear cell carcinoma, showed discordant expression patterns between the primary and metastatic lesions. In conclusion, this study demonstrated variable TROP2 expression in brain metastasis samples and confirmed high consistency of TROP2 expression between primary and brain metastatic lesions.

Keywords: TROP2, brain metastasis, immunohistochemistry, cancer, primary tumor

Introduction

The human trophoblast cell surface antigen 2 (TROP2) is a transmembrane glycoprotein that acts as an intracellular calcium signal transducer, driving self-renewal, cell proliferation, invasion, and survival [1, 2]. It is overexpressed in various solid tumors, including breast [3], Colorectal [4, 5], lung [6], gastric [7], skin [8], and pancreatic cancers [9], with expression reported in 55-75% of cases. Overexpression of TROP2 is generally associated with poor prognosis, increased tumor aggressiveness, and higher metastatic potential [10, 11].

Metastatic solid tumors remain a significant unmet medical challenge, particularly central nervous system (CNS) metastases, which are

associated with extremely poor outcomes. While targeted therapies, such as monoclonal antibodies and antibody-drug conjugates (ADCs), have improved outcomes in advanced cancer, their efficacy against CNS metastases is limited. Sacituzumab govitecan (SG), a TROP2-directed ADC, has demonstrated significant overall survival (OS) benefits in patients with triple-negative breast cancer (TNBC) [12] but showed no OS improvement for those with CNS metastases [13]. The prevailing explanation attributes this limitation to the inability of these therapies to effectively penetrate the blood-brain barrier (BBB) [14]. However, the distinct molecular and genomic features of CNS metastases, which often diverge from those of the primary tumor, must also be considered [15].

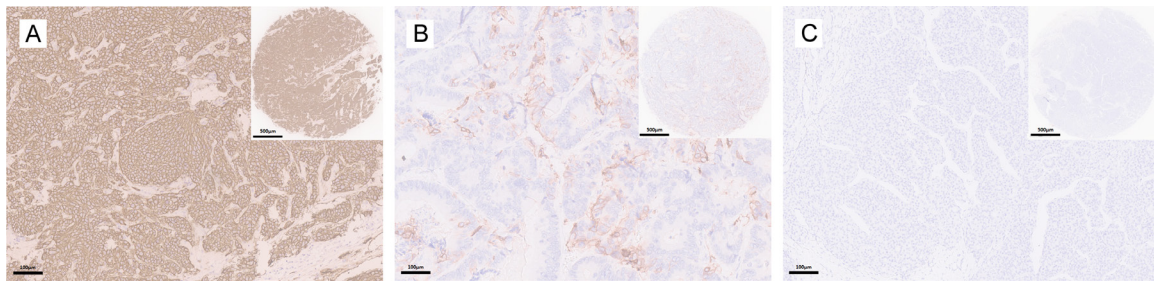


Figure 1. Representative images of TROP2 immunohistochemical (IHC) staining in metastatic brain tumor. A. Metastatic invasive breast carcinoma, no special type showing diffuse TROP2 positivity ($\times 200$, $\times 40$ inlet). B. Metastatic adenocarcinoma from the colon showing TROP2 focal positivity ($\times 400$, $\times 40$ inlet). C. Metastatic follicular carcinoma from the thyroid gland showing TROP2 negativity ($\times 200$, $\times 40$ inlet).

Research on TROP2 heterogeneity between primary and metastatic lesions, particularly in the CNS, remains scarce. Based on this context, this study aims to evaluate TROP2 expression in brain metastases from various solid tumors and compare it with expression in primary tumors.

Materials and methods

Case selection

After approval from the institutional review board (approval number: 2024AN0580), a total of 61 surgically resected brain metastasis specimens between 2015 to 2023 were retrieved from the pathology database of the Department of Pathology at the Anam Hospital, Korea University College of Medicine, Seoul, Republic of Korea. When available, corresponding primary tumor tissues were also collected. Clinical data, including the patient's age, sex, origin of tumor was reviewed in the electronic medical records.

Tissue microarray construction

Tissue microarrays (TMAs) were constructed from formalin-fixed, paraffin-embedded tissue blocks by using a manual tissue micro-arrayer (UNITIMA, Seoul, South Korea). Areas occupied for $> 75\%$ of tumor cells without accompanying tumor necrosis were selected and two representative cores were punched out from a donor block and transferred into a new recipient block with a punch of a 3-mm diameter.

Immunohistochemical staining

The Immunohistochemical (IHC) staining was performed as follows. Briefly, 4- μm tissue sec-

tions from TMAs were subjected to IHC using a Ventana auto-stainer and an ultra-Vies DAB Detection Kit (Ventana, Tucson, Arizona), according to the manufacturer's instruction. Primary antibodies for TROP2 (EPR20043, catalog No. ab214488, rabbit monoclonal, 1:2000, Abcam, Cambridge, UK) were used. The TROP2 IHC staining was evaluated by two pathologists (YNS and HO) and classified into 3 categories (**Figure 1**): Diffuse positive ($> 95\%$), focal positive ($> 0\%$ and $< 95\%$), and negative.

Statistical methods

The R software (version 4.02, Vienna, Austria) was used. The association between TROP2 expression pattern and tumor origin, as well as between TROP2 expression pattern and pathologic diagnosis, was evaluated using the χ^2 and/or Fisher's exact tests. Survival analysis based on expression levels was performed using log rank test and Kaplan-Meier analysis. *P*-values less than 0.05 were considered statistically significant.

Results

Clinicopathological characteristics of cases

A total of 61 surgically resected brain metastasis specimens were available for analysis. **Table 1** summarizes the characteristics of cases. The mean age of the patients was 61.5 ± 10.0 years (range, 37 to 82 years) with a male-to-female ratio of 1.1. Metastatic brain tumors originating from various organs were included. The most common primary site was lung (23 cases, 37.7%), followed by the breast (11 cases, 18.0%) and the colorectum (10 cases, 16.4%).

Table 1. Clinicopathological characteristics of the analyzed cases

Characteristics	No. of patients	% of patients
Age (years)	61.5±10.0	
Sex		
Male	32	52.5
Female	29	47.5
Primary site		
Lung	23	37.7
Breast	11	18.0
Colorectal	10	16.4
Bladder	3	4.9
Skin	3	4.9
Kidney	2	3.3
Ovary	2	3.3
Unknown	2	3.3
Ear	1	1.6
Gallbladder	1	1.6
Hypopharynx	1	1.6
Thymus	1	1.6
Thyroid	1	1.6
Diagnosis		
Adenocarcinoma	27	44.3
Invasive ductal carcinoma	11	18.0
Squamous cell carcinoma	6	9.8
Carcinoma, undifferentiated	4	6.6
Melanoma	3	4.9
Urothelial carcinoma	3	4.9
Clear cell carcinoma	2	3.3
High-grade serous carcinoma	2	3.3
Adenoid cystic carcinoma	1	1.6
Follicular carcinoma	1	1.6
Thymoma	1	1.6
Median time to brain metastasis (months, range)	23.5 (0-137)	
Median overall survival (months, range)	40.3 (5-98)	
Median survival post-CNS metastasis (months, range)	9.9 (0-76)	
CNS metastasis number		
Single	37	60.7
Multiple	24	39.3
CNS metastasis treatment		
Surgery	61	100.0
Radiotherapy	31	50.8

Other primary sites included the bladder and skin (3 cases each, 4.9%), kidney and ovary (2 cases each, 3.3%), as well as less common sites such as the ear, gallbladder, hypopharynx, thymus, and thyroid, each contributing 1 case (1.6%). In terms of pathologic diagnosis, adeno-

carcinoma was the most frequently observed type, with 27 cases (44.3%), followed by invasive ductal carcinoma with 11 cases (18.0%), Squamous cell carcinoma with 6 cases (9.8%), and undifferentiated carcinoma in 4 cases (6.6%). Melanoma and urothelial carcinoma were found in 3 cases each (4.9%). Less common diagnoses included clear cell carcinoma and high-grade serous carcinoma (2 cases each, 3.3%), along with adenoid cystic carcinoma, follicular carcinoma, and thymoma each present in 1 case (1.6%).

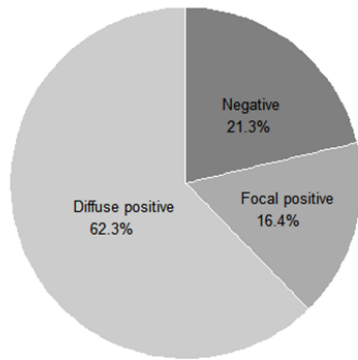
TROP2 expression in metastatic and primary tumor

Among the 61 metastatic brain tumors, TROP2 expression was diffusely positive in 38 cases (62.3%), focally positive in 10 cases (16.4%), and negative in 13 cases (21.3%, **Figure 2A**). Of the tumors with diffuse positive TROP2 expression, the most common site of origin was the lung (18 cases, 47.4%), followed by the breast (11 cases, 28.9%), bladder (3 cases, 7.9%), and ovary (2 cases, 5.3%). Single cases originated from colorectal, ear, gallbladder, and hypopharyngeal primaries. Among the tumors with focal TROP2 expression, 8 cases (80%) originated from colorectum, and 2 cases (20%) were from the lung. In the TROP2-negative cases, lung and skin tumors accounted for 3 cases each (23.1%), kidney and tumors of unknown origin accounted for

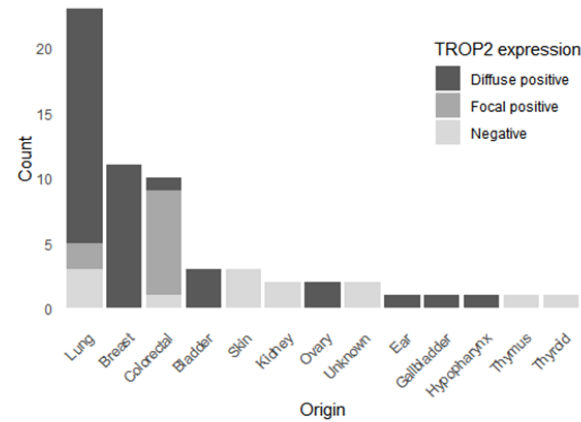
2 cases each (15.4%), and colorectal, thyroid, and thymic tumors each accounted for 1 case (7.7%, **Figure 2B**). The distribution of TROP2 expression according to pathologic diagnosis is illustrated in **Figure 2C**. Notably, in the most common tumor type, adenocarcinoma, TROP2

TROP2 expressions in brain metastatic and primary tumors

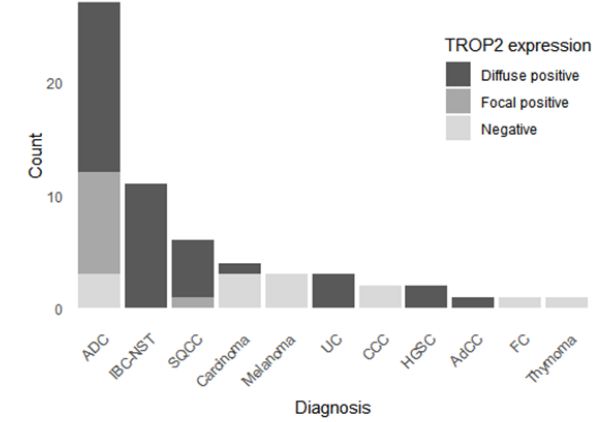
A TROP2 expression in metastatic brain tumor



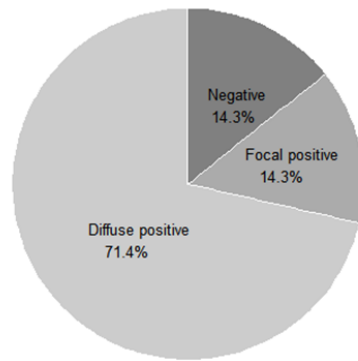
B TROP2 expression in brain metastasis according to origin



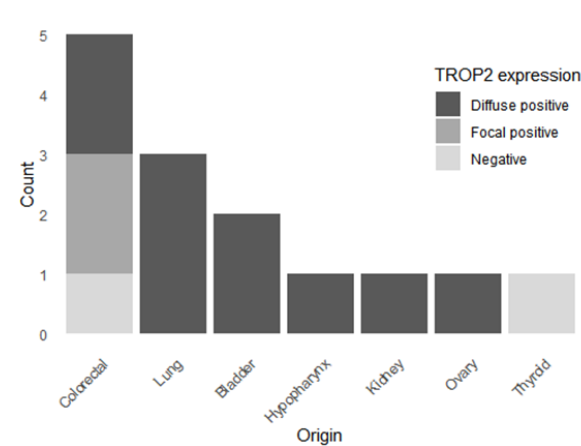
C TROP2 expression in brain metastasis according to diagnosis



D TROP2 expression in primary tumor



E TROP2 expression in primary tumor according to origin



F TROP2 expression in primary tumor according to diagnosis

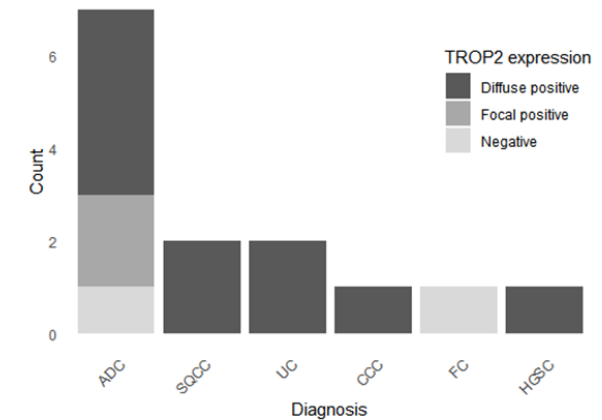


Figure 2. TROP2 expression in metastatic and primary brain tumors. A. Pie chart showing TROP2 expression pattern in metastatic brain tumors. B, C. Bar chart showing TROP2 expression in metastatic brain tumor according to origin and diagnosis. D. Pie chart showing TROP2 expression pattern in primary tumors. E, F. Bar chart showing TROP2 expression in primary tumor according to origin and diagnosis.

TROP2 expressions in brain metastatic and primary tumors

Table 2. TROP2 expression according to origin in brain metastasis and primary tumor

Origin	TROP2 expression			p-value
	Diffuse positive	Focal positive	Negative	
Brain metastasis (n = 61)				
Lung	18 (47.4%)	2 (20%)	3 (23.1%)	P < 0.001
Breast	11 (28.9%)	0 (0%)	0 (0%)	
Colorectal	1 (2.6%)	8 (80%)	1 (7.7%)	
Bladder	3 (7.9%)	0 (0%)	0 (0%)	
Skin	0 (0%)	0 (0%)	3 (23.1%)	
Kidney	0 (0%)	0 (0%)	2 (15.4%)	
Ovary	2 (5.3%)	0 (0%)	0 (0%)	
Unknown	0 (0%)	0 (0%)	2 (15.4%)	
Ear	1 (2.6%)	0 (0%)	0 (0%)	
Gallbladder	1 (2.6%)	0 (0%)	0 (0%)	
Hypopharynx	1 (2.6%)	0 (0%)	0 (0%)	
Thymus	0 (0%)	0 (0%)	1 (7.7%)	
Thyroid	0 (0%)	0 (0%)	1 (7.7%)	
Total	38	10	13	
Primary tumor (n = 14)				
Colorectal	2 (20%)	2 (100%)	1 (50%)	P = 0.420
Lung	3 (30%)	0 (0%)	0 (0%)	
Bladder	2 (20%)	0 (0%)	0 (0%)	
Hypopharynx	1 (10%)	0 (0%)	0 (0%)	
Kidney	1 (10%)	0 (0%)	0 (0%)	
Ovary	1 (10%)	0 (0%)	0 (0%)	
Thyroid	0 (0%)	0 (0%)	1 (50%)	
Total	1	2	2	

expression was diffusely positive in 15 cases (55.6%), focally positive in 9 cases (33.3%), and negative in 3 cases (11.1%). In contrast, all 11 cases of invasive breast carcinoma of no special type (IBC-NST) showed diffuse TROP2 positivity. Similarly, in primary tumors, TROP2 expression was diffusely positive in 10 cases (71.4%), focally positive in 2 cases (14.3%), and negative in 2 cases (14.3%, **Figure 2D**). Among the TROP2 diffuse positive cases, 3 cases (30%) originated from the lung, 2 cases (20%) each from the bladder and colorectum, and 1 case (10%) each from the hypopharynx, kidney, and ovary. For the focal TROP2 positive cases, both originated from colorectal primaries (100%). Among the TROP2-negative cases, 1 case (50%) originated from the colorectum and 1 case (50%) from the thyroid (**Figure 2E**). The distribution of TROP2 expression by pathologic diagnosis showed a pattern similar to that of brain metastatic lesions, with adenocarcino-

ma being the most common tumor type (**Figure 2F**). In adenocarcinomas, TROP2 expression was diffusely positive in 4 cases (57.1%), focally positive in 2 cases (28.6%), and negative in 1 case (14.3%). All cases of squamous cell carcinoma, urothelial carcinoma, clear cell carcinoma, and high-grade serous carcinoma demonstrated diffuse TROP2 positivity, while follicular carcinoma was TROP2-negative. Detailed TROP2 expression according to tumor origin and pathologic diagnosis for both brain metastatic lesions and primary tumors are provided in **Tables 2** and **3**.

Differences in TROP2 expression based on clinical characteristics

We analyzed whether differences in TROP2 expression were associated with characteristics of CNS metastases or patient survival. Patients were categorized into two groups based on the presentation of CNS metastases at

the time of diagnosis: single vs. multiple metastases.

When stratified by TROP2 expression levels, the distribution was as follows: among patients with diffuse positive expression, 21 had single CNS metastasis, and 16 had multiple metastases. In the focal positive group, 8 had single CNS metastasis, and 2 had multiple metastases. Among patients with negative expression, 7 had single CNS metastasis, and 6 had multiple metastases. Fisher's exact test did not reveal a significant association ($P = 0.454$).

The median survival post-CNS metastasis was: diffuse positive, 76.4 months (95% CI: 46.8-NA); focal positive, 18.5 months (95% CI: 13.5-NA); and negative, 43.0 months (95% CI: 19.0-NA). The log-rank test again showed no significant differences ($P = 0.218$). HRs were 2.93 (95% CI: 0.72-11.94, $P = 0.134$) for focal positive relative to diffuse positive, and 1.97

TROP2 expressions in brain metastatic and primary tumors

Table 3. TROP2 expression according to diagnosis in brain metastasis and primary tumor

Origin	TROP2 expression			p-value
	Diffuse positive	Focal positive	Negative	
Brain metastasis (n = 61)				
Adenocarcinoma	15 (39.5%)	9 (90%)	3 (23.1%)	P < 0.001
Invasive breast carcinoma-no special type	11 (28.9%)	0 (0%)	0 (0%)	
Squamous cell carcinoma	5 (13.2%)	1 (10%)	0 (0%)	
Carcinoma	1 (2.6%)	0 (0%)	3 (23.1%)	
Melanoma	0 (0%)	0 (0%)	3 (23.1%)	
Urothelial carcinoma	3 (7.9%)	0 (0%)	0 (0%)	
Clear cell carcinoma	0 (0%)	0 (0%)	2 (15.4%)	
High-grade serous carcinoma	2 (5.3%)	0 (0%)	0 (0%)	
Adenoid cystic carcinoma	1 (2.6%)	0 (0%)	0 (0%)	
Follicular carcinoma	0 (0%)	0 (0%)	1 (7.7%)	
Thymoma	0 (0%)	0 (0%)	1 (7.7%)	
Total	38	10	13	
Primary tumor (n = 14)				
Adenocarcinoma	4 (40%)	2 (100%)	1 (50%)	P = 0.476
Squamous cell carcinoma	2 (20%)	0 (0%)	0 (0%)	
Urothelial carcinoma	2 (20%)	0 (0%)	0 (0%)	
Clear cell carcinoma	1 (10%)	0 (0%)	0 (0%)	
Follicular carcinoma	0 (0%)	0 (0%)	1 (50%)	
High-grade serous carcinoma	1 (10%)	0 (0%)	0 (0%)	
Total	10	2	2	

(95% CI: 0.64-6.08, P = 0.237) for negative relative to diffuse positive (**Figure 3A**).

Given that only 17 death events occurred while 44 patients were censored, which may have led to an overestimation of survival in the Kaplan-Meier analysis, we additionally performed a descriptive survival analysis. In this analysis, the median survival after CNS metastasis was 38.7 months (95% CI: 30.6-76.4) in the diffuse positive group, 12.0 months (95% CI: 3.8-NA) in the focal positive group, and 23.6 months (95% CI: 3.0-NA) in the negative group. The log-rank test indicated statistically significant differences among the groups (P < 0.001). The hazard ratios were 5.75 (95% CI: 2.38-13.89, P < 0.001) for focal positive relative to diffuse positive and 1.80 (95% CI: 0.82-3.95, P = 0.144) for negative relative to diffuse positive (**Figure 3B**).

Comparison of TROP2 expression in paired primary and brain metastatic tumor

Among the 14 cases where both primary tumors and corresponding brain metastases were available for paired TROP2 expression

analysis, three cases (21.4%) showed differences in TROP2 expression between the primary and metastatic sites (**Figure 4; Table 4**). Specifically, in colorectal adenocarcinoma, one case shifted from diffuse positive in the primary lesion to focal positive in the metastasis, and another case showed negative expression in the primary lesion but focal positivity in the metastasis. Additionally, a case of kidney clear cell carcinoma exhibited diffuse positive TROP2 expression in the primary tumor but was negative in the brain metastasis. The remaining 11 cases (78.6%) displayed consistent TROP2 expression patterns between the primary and metastatic lesions.

Discussion

This study provides important insights into the TROP2 expression in brain metastases across various primary tumor origins. Notably, the high prevalence of diffuse TROP2 positivity (62.3%) in brain metastases, particularly those originating from lung and breast cancers, highlights the potential role of TROP2 in the pathogenesis and progression of CNS metastasis.

TROP2 expressions in brain metastatic and primary tumors

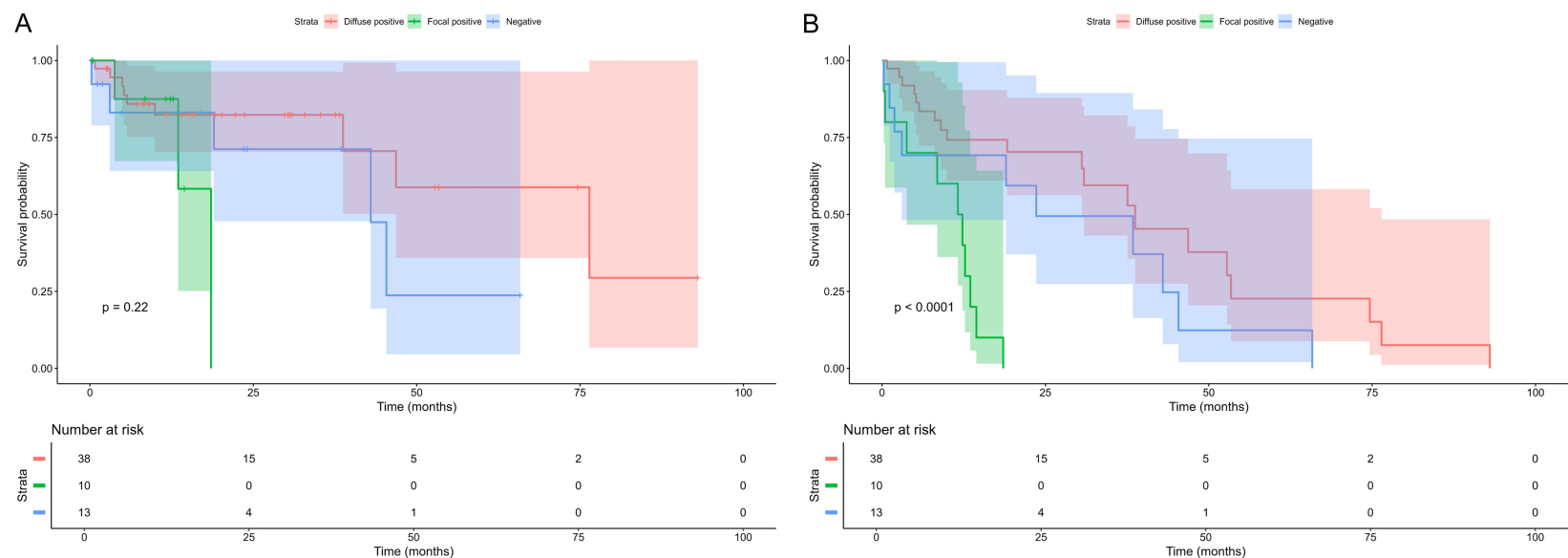


Figure 3. Post-CNS survival stratified by TROP2 expression levels. A. Kaplan-Meier survival analysis. B. Descriptive survival analysis. Median values with 95% confidence intervals are presented. 'NA' indicates that the upper bound of the confidence interval was not available due to limited events.

TROP2 expressions in brain metastatic and primary tumors

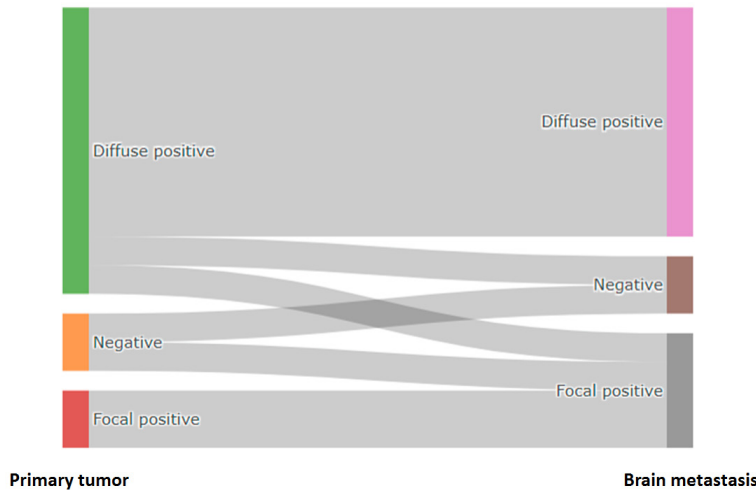


Figure 4. Sankey diagram illustrating the flow of TROP2 expression between primary and metastatic brain tumors.

Anti-cancer therapies targeting TROP2 have predominantly focused on the development of ADC. A representative TROP2 ADC, sacituzumab govitecan (SG), consists of a TROP2-targeting antibody linked to the topoisomerase I inhibitor SN-38 via a cleavable linker, with a drug-antibody ratio of 1:8, demonstrating potent anti-cancer effects [16]. In the ASCENT phase 3 clinical trial, SG significantly improved median progression-free survival (mPFS; HR 0.41, 95% CI 0.32-0.52, $P < 0.001$) and median overall survival (mOS; HR 0.48, 95% CI 0.38-0.59, $P < 0.001$) in patients with advanced TNBC [12], leading to FDA approval. In the TROPICS-02 trial, targeting HR+/HER2- breast cancer, SG also demonstrated a significant mOS benefit compared to the control group (HR 0.79, 95% CI 0.65-0.96, $P = 0.020$) [17]. Additionally, SG has shown therapeutic potential in minor cancer types, such as endometrial cancer, small cell lung cancer, and castration-resistant prostate cancer [18]. Based on these findings, numerous combination therapy clinical trials are currently underway [19].

Datopotamab deruxtecan (Dato-DXd) is another ADC that combines a TROP2 antibody with DXd, featuring a drug-to-antibody ratio of 1:4 [20]. In HR+/HER2- breast cancer, it demonstrated a significant improvement in mPFS compared to the control group (HR 0.63, 95% CI 0.52-0.76, $P < .0001$) [21]. In a phase 3 clinical trial for patients with advanced NSCLC, it also showed a significant mPFS benefit over docetaxel (HR 0.75, 95% CI 0.62-0.91, $P =$

.004) [22]. Promising results have recently been reported in endometrial and ovarian cancer cohorts [23]. Additionally, other TROP2-targeting ADCs, such as SKB264, SHR-A1921, and LCB84, are undergoing early clinical trials based on encouraging preclinical data [24-26].

Preclinical studies suggest a potential correlation between TROP2 expression and the efficacy of ADCs. In breast cancer cell lines, increased TROP2 expression was associated with enhanced therapeutic activity of SG [27]. Similarly, clinical trials targeting TNBC

demonstrated the greatest efficacy in patients with medium to high TROP2 expression, with objective response rates of 44%, 38%, and 22% for high, medium, and low expression levels, respectively [28]. However, clinical data across various cancer types remain limited, and due to the bystander effect of ADCs and the heterogeneity of TROP2 expression, definitive conclusions cannot yet be drawn.

As cancer survival improves, recurrent CNS metastases following local treatment have become increasingly common, highlighting the need for effective systemic therapies. In cancers with high rates of brain metastases, such as breast and lung cancer, clinical trials now frequently include CNS-specific endpoints. Systemic treatment for CNS metastases remains challenging due to the BBB. While small molecules like TKIs have demonstrated CNS efficacy [29, 30], larger agents like SG (160 kDa) face limited BBB penetration [31]. However, local BBB disruptions from metastases or radiotherapy may enable larger molecules to access CNS lesions, making target expression a critical determinant of efficacy. Our study supports this concept, with 78.7% of 61 brain metastases exhibiting diffuse or focal TROP2 expression. Consistency in TROP2 expression between primary and CNS lesions was observed in 78.6% of matched cases. These findings suggest a potential association between TROP2 expression and the efficacy of anti-TROP2 ADCs in CNS metastases, warranting further investigation.

TROP2 expressions in brain metastatic and primary tumors

Table 4. Comparison of TROP2 expression in paired primary and metastatic brain tumors

Origin	Diagnosis	TROP2 expression	
		Primary	Metastasis
Colorectal	Adenocarcinoma	Diffuse positive	Focal positive
Colorectal	Adenocarcinoma	Focal positive	Focal positive
Colorectal	Adenocarcinoma	Diffuse positive	Diffuse positive
Colorectal	Adenocarcinoma	Focal positive	Focal positive
Colorectal	Adenocarcinoma	Negative	Focal positive
Lung	Adenocarcinoma	Diffuse positive	Diffuse positive
Lung	Adenocarcinoma	Diffuse positive	Diffuse positive
Lung	Squamous cell carcinoma	Diffuse positive	Diffuse positive
Bladder	Urothelial carcinoma	Diffuse positive	Diffuse positive
Bladder	Urothelial carcinoma	Diffuse positive	Diffuse positive
Hypopharynx	Squamous cell carcinoma	Diffuse positive	Diffuse positive
Kidney	Clear cell carcinoma	Diffuse positive	Negative
Ovary	High-grade serous carcinoma	Diffuse positive	Diffuse positive
Thyroid	Follicular carcinoma	Negative	Negative

This study represents the largest cohort of brain metastases evaluated for TROP2 expression using standardized IHC. All staining was performed in a single center, with a single pathologist ensuring consistency, and the analysis was blinded to treatment data to minimize bias. Previous studies evaluating TROP2 expression in various cancers, including breast [3, 32, 33], colorectal [4, 5], skin [8, 34], and pancreas [9], primarily used semi-quantitative assessment methods such as H-score to evaluate TROP2 expression levels. These studies often relied on specific scoring thresholds to categorize TROP2 expression. In contrast, our study demonstrated a relatively clear differentiation of TROP2 expression into diffuse, focal, and negative patterns, allowing for straightforward analysis without the need for further intensity or area-based categorization. It is important to note that factors such as the type of antibody used and the application of tissue microarrays (TMAs) may have influenced the observed TROP2 expression patterns. While TMAs are valuable for high-throughput analysis, they may not fully capture the tumor heterogeneity present in whole-tumor sections. Therefore, future studies examining cases with changes in TROP2 expression between primary and metastatic lesions, or those showing focal positivity, would benefit from evaluating TROP2 expression using whole-slide sections to better reflect the full spectrum of tumor heterogeneity.

In our analysis, the large proportion of censored cases led to an overestimation of survival in the Kaplan-Meier analysis. To better reflect the clinical situation, we therefore incorporated descriptive survival analysis. The focal positive group showed the poorest survival, whereas the diffuse positive and negative groups did not follow this pattern, suggesting that a stepwise relationship between TROP2 expression and prognosis could not be established. Given the relatively small sample size, single-center setting, and retrospective nature of the study, these findings should be interpreted with caution, and confirmatory studies with larger, prospective, multi-institutional cohorts are warranted.

Comprehensive clinical data enabled robust correlations with TROP2 expression. However, the inclusion of diverse tumor types limited subgroup analyses, and the lack of data from visceral metastases prevents conclusions about the specificity of TROP2 expression to CNS lesions. Furthermore, as samples were collected before TROP2 ADCs became available, intracranial efficacy based on TROP2 expression could not be assessed. Moreover, the discrepancy between Kaplan-Meier and descriptive survival analyses, largely due to the high proportion of censored cases, should be interpreted with caution. Further studies are required to validate these findings.

In conclusion, this study demonstrated variable TROP2 expression in 61 brain metastasis samples and confirmed high consistency of TROP2 expression between primary and brain metastatic lesions. While promising, further research is required to validate the clinical efficacy of anti-TROP2 therapies in patients with CNS metastases.

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Disclosure of conflict of interest

None.

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References

- [1] Shvartsur A and Bonavida B. Trop2 and its overexpression in cancers: regulation and clinical/therapeutic implications. *Genes Cancer* 2015; 6: 84-105.
- [2] Ripani E, Sacchetti A, Corda D and Alberti S. Human Trop-2 is a tumor-associated calcium signal transducer. *Int J Cancer* 1998; 76: 671-676.
- [3] Ambroggi F, Fornili M, Boracchi P, Trerotola M, Relli V, Simeone P, La Sorda R, Lattanzio R, Quersoli P, Pedriali M, Piantelli M, Biganzoli E and Alberti S. Trop-2 is a determinant of breast cancer survival. *PLoS One* 2014; 9: e96993.
- [4] Moretto R, Germani MM, Giordano M, Conca V, Proietti A, Niccoli C, Pietrantonio F, Lonardi S, Tamburini E, Zaniboni A, Passardi A, Latiano TP, Fanotto V, Di Donato S, Prisciandaro M, Bergamo F, Masi G, Fontanini G, Ugolini C and Cremolini C. Trop-2 and Nectin-4 immunohistochemical expression in metastatic colorectal cancer: searching for the right population for drugs' development. *Br J Cancer* 2023; 128: 1391-1399.
- [5] Foersch S, Schmitt M, Litmeyer AS, Tschurtschenthaler M, Gress T, Bartsch DK, Pfarr N, Steiger K, Denkert C and Jesinghaus M. TROP2 in colorectal carcinoma: associations with histopathology, molecular phenotype, and patient prognosis. *J Pathol Clin Res* 2024; 10: e12394.
- [6] Lin JC, Wu YY, Wu JY, Lin TC, Wu CT, Chang YL, Jou YS, Hong TM and Yang PC. TROP2 is epigenetically inactivated and modulates IGF-1R signalling in lung adenocarcinoma. *EMBO Mol Med* 2012; 4: 472-485.
- [7] Zhao W, Zhu H, Zhang S, Yong H, Wang W, Zhou Y, Wang B, Wen J, Qiu Z, Ding G, Feng Z and Zhu J. Trop2 is overexpressed in gastric cancer and predicts poor prognosis. *Oncotarget* 2016; 7: 6136-6145.
- [8] Ito T, Tanegashima K, Tanaka Y, Hashimoto H, Murata M, Oda Y and Kaku-Ito Y. Trop2 expression in extramammary Paget's disease and normal skin. *Int J Mol Sci* 2021; 22: 7706.
- [9] Fong D, Moser P, Krammel C, Gostner JM, Margreiter R, Mitterer M, Gastl G and Spizzo G. High expression of TROP2 correlates with poor prognosis in pancreatic cancer. *Br J Cancer* 2008; 99: 1290-1295.
- [10] Zeng P, Chen MB, Zhou LN, Tang M, Liu CY and Lu PH. Impact of TROP2 expression on prognosis in solid tumors: a systematic review and meta-analysis. *Sci Rep* 2016; 6: 33658.
- [11] Cubas R, Li M, Chen C and Yao Q. Trop2: a possible therapeutic target for late stage epithelial carcinomas. *Biochim Biophys Acta* 2009; 1796: 309-314.
- [12] Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, Brufsky A, Sardesai SD, Kalinsky K, Zelnak AB, Weaver R, Traina T, Dalenc F, Aftimos P, Lynce F, Diab S, Cortés J, O'Shaughnessy J, Diéras V, Ferrario C, Schmid P, Carey LA, Gianni L, Piccart MJ, Loibl S, Goldenberg DM, Hong Q, Olivo MS, Itri LM and Rugo HS; ASCENT Clinical Trial Investigators. Sacituzumab govitecan in metastatic triple-negative breast cancer. *N Engl J Med* 2021; 384: 1529-1541.
- [13] Diéras V, Weaver R, Tolaney SM, Bardia A, Punie K, Brufsky A, Rugo HS, Kalinsky K, Traina T, Klein L, Loirat D, Lynce F, Daniel B, Ademuyiwa F, Hurvitz SA, Goldenberg DM, Hong Q, Olivo M, Itri LM and Carey L. Abstract PD13-07: subgroup analysis of patients with brain metastases from the phase 3 ASCENT study of sacituzumab govitecan versus chemotherapy in metastatic triple-negative breast cancer. *Cancer Res* 2021; 81: PD13-07-PD13-07.
- [14] Upton DH, Ung C, George SM, Tsoli M, Kavaliris M and Ziegler DS. Challenges and opportunities to penetrate the blood-brain barrier for brain cancer therapy. *Theranostics* 2022; 12: 4734-4752.

- [15] Ali S, Górská Z, Duchnowska R and Jassem J. Molecular profiles of brain metastases: a focus on heterogeneity. *Cancers (Basel)* 2021; 13: 2645.
- [16] Goldenberg DM and Sharkey RM. Sacituzumab govitecan, a novel, third-generation, antibody-drug conjugate (ADC) for cancer therapy. *Expert Opin Biol Ther* 2020; 20: 871-885.
- [17] Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, Trédan O, Ciruelos E, Dalenc F, Gómez Pardo P, Jhaveri KL, Delaney R, Valdez T, Wang H, Motwani M, Yoon OK, Verret W and Tolaney SM. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPICS-02): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2023; 402: 1423-1433.
- [18] Bardia A, Messersmith WA, Kio EA, Berlin JD, Vahdat L, Masters GA, Moroosse R, Santin AD, Kalinsky K, Picozzi V, O'Shaughnessy J, Gray JE, Komiya T, Lang JM, Chang JC, Starodub A, Goldenberg DM, Sharkey RM, Maliakal P, Hong Q, Wegener WA, Goswami T and Ocean AJ. Sacituzumab govitecan, a Trop-2-directed antibody-drug conjugate, for patients with epithelial cancer: final safety and efficacy results from the phase I/II IMMUNO-132-01 basket trial. *Ann Oncol* 2021; 32: 746-756.
- [19] Garon EB, Liu SV, Owen SP, Reck M, Neal JW, Vicente D, Mekan SF, Safavi F, Fernando N and Mok TSK. EVOKE-02: a phase 2 study of sacituzumab govitecan (SG) plus pembrolizumab (pembro) with or without platinum chemotherapy in first-line metastatic non-small cell lung cancer (NSCLC). *J Clin Oncol* 2022; 40: TPS9146.
- [20] Okajima D, Yasuda S, Maejima T, Karibe T, Sakurai K, Aida T, Toki T, Yamaguchi J, Kitamura M, Kamei R, Fujitani T, Honda T, Shibutani T, Muramatsu S, Nakada T, Goto R, Takahashi S, Yamaguchi M, Hamada H, Noguchi Y, Murakami M, Abe Y and Agatsuma T. Datopotamab deruxtecan, a novel TROP2-directed antibody-drug conjugate, demonstrates potent antitumor activity by efficient drug delivery to tumor cells. *Mol Cancer Ther* 2021; 20: 2329-2340.
- [21] Bardia A, Jhaveri K, Im SA, Pernas S, De Laurentiis M, Wang S, Martínez Jañez N, Borges G, Cescon DW, Hattori M, Lu YS, Hamilton E, Zhang Q, Tsurutani J, Kalinsky K, Rubini Liedke PE, Xu L, Fairhurst RM, Khan S, Denduluri N, Rugo HS, Xu B, Pistilli B, Romitelli B, Korbenfeld E, Buono C, Barbero A, Rosselli G, Daniele S, Ostoich SA, Wildiers H, Punie K, Collignon J, Jerusalem G, Gombos A, Borges G, Liedke PER, Cesca M, Beato P, Testa L, Pinczowski H, Rapatoni L, Jardim D, Bines J, Cescon DW, Aselah J, Blais A, Yu J, Friedmann J, Ferrario C, Xu B, Wang S, Zhang Q, Jiang Z, Tong Z, Ouyang Q, Wang J, Yao T, Wang Y, Wang X, Sun M, Li H, Wang S, Sheng Y, Zang A, Zhang Z, Chen W, Wang X, Ouyang Z, Li W, Pistilli B, Bachelot T, Ung M, Villanueva C, Garbay D, Hardy-Bessard AC, Mailliez A, Becourt S, Mina W, Decker T, Radosa J, Schneeweiss A, Braun M, Aktas B, Rubovszky G, Pápai Z, Csösz T, Al-Farhat Y, Patel A, Gupta V, Sharma R, Venkatesha CM, Bondarde S, Roy S, Ghadyalpatil N, Sharma L, Yedla R, Laurentiis MD, Paris I, Zagni C, Guarnieri V, Meattini I, Colleoni M, Bianchini G, Giorgi UD, Montemurro F, Geuna E, Biganzoli L, Tsurutani J, Hattori M, Ozaki Y, Shimomura A, Niikura N, Itoh M, Taira T, Mukohara T, Aogi K, Iwasa T, Tokunaga E, Saji S, Kawaguchi N, Yamashita T, Inoue K, Nakayama T, Watanabe K, Nagahashi M, Yonemori K, Drooger J, Konings I, Wouw Avd, Nowecki Z, Chmielowska E, Danielewicz I, Jassem J, Kalinka E, Czartoryska-Arlukowicz B, Karaszewska B, Kwiatkowski M, Im SA, Sohn JH, Park YH, Lee KS, Jung KH, Park KH, Kim JH, Stroyakovskiy D, Artamonova E, Mekebebe-Reuter M, Rapoport B, Schoeman E, Coccia-Portugal M, Mathiba R, Pernas S, Jañez NM, Adamo B, Heras BBdl, Sáenz JÁG, Borrego MR, Domínguez ME, Rodríguez BJ, Novoa SA, Calvo EG, Castán JC, Calero JLB, Chen SC, Tseng LM, Lu YS, Chung WP, Chang YC, Liu CT, Wang HC, Rau KM, Comins C, Braybrooke J, Borley A, O'Brien C, Michie C, Schmid P, McGrath S, Wheatley D, Mukesh M, Trivedi S, Karim S, Bezecny P, Bardia A, Rugo HS, Kalinsky K, Hamilton E, Jhaveri K, Yuan Y, Patel N, Mortimer J, Tolaney S, Woude AV, Wright G, Riaz F, Pandey A, Moore H, Ross M and McCann K. Datopotamab deruxtecan versus chemotherapy in previously treated inoperable/metastatic hormone receptor-positive human epidermal growth factor receptor 2-negative breast cancer: primary results from TROPION-Breast01. *J Clin Oncol* 2025; 43: 285-296.
- [22] Ahn MJ, Tanaka K, Paz-Ares L, Cornelissen R, Girard N, Pons-Tostivint E, Vicente Baz D, Sugawara S, Cobo M, Pérol M, Mascaux C, Poddubskaya E, Kitazono S, Hayashi H, Hong MH, Felipe E, Hall R, Juan-Vidal O, Brungs D, Lu S, Garassino M, Chargualaf M, Zhang Y, Howarth P, Uema D, Lisberg A, Sands J, Martinengo GL, Puig J, Brungs D, Gao B, Nagria A, Karapetis C, Parakh S, Park J, Catala G, Forget F, Ocak S, Basappa N, Liu G, Menjak I, Shieh B, Lu S, Luo F, Sun H, Wang J, Yao Y, Zemanova M, Benouna J, Girard N, Greillier L, Lamour C, Lena H, Mascaux C, Mazieres J, Moro-Sibilot D, Pérol M, Pons-Tostivint E, Westeel V, Atmaca A, Greil C, Reinmuth N, Schumann C, Wehler T, Wolf J,

- Ho J, Bocskai C, Cappuzzo F, Marinis FD, Proto C, Mencoboni M, Novello S, Salvagni S, Parra HS, Daga H, Goto Y, Hayashi H, Kitazono S, Yoh K, Ko R, Kondo M, Kozuki T, Kurata T, Mizutani H, Ohashi K, Oizumi S, Okamoto I, Sakaguchi S, Ozasa H, Sugawara S, Tambo Y, Tamiya M, Tanaka H, Okamoto I, Lopez-Lopez F, Godinez FJR, Cid JRR, Cornelissen R, Gans S, Stigt J, Kowalski D, Lowczak A, Milanowski J, Mróz R, Ramlau R, Acosta-Rivera M, Ahn MJ, Chae YS, Hong MH, Kang JH, Kim SW, Kim JS, Kim SH, Lee KH, Lee YG, Shim BY, Ledin E, Poddubska E, Chan BYD, Jain A, Tan CS, Areses MC, Cobo M, Domine M, Felip E, Pradera JF, Isla D, Vidal OJ, Majem M, Paz-Ares L, Provencio M, Reguart N, Baz DV, Cerciello F, Früh M, Chang WC, Chang GC, Huang WT, Ling CC, Wei YF, Yang TY, Ahmad T, Gomes F, Mansy T, Bruno D, Castine M, Fang B, Garassino M, Hall R, Halmos B, Jahangir K, Johnson M, Johnson T, Kalmadi S, Lawler W, Lisberg A, Sadiq A, Sands J, Solomon B and Veatch A. Datopotamab derux-tecan versus docetaxel for previously treated advanced or metastatic non-small cell lung cancer: the randomized, open-label phase III TROPION-Lung01 study. *J Clin Oncol* 2025; 43: 260-272.
- [23] Oaknin A, Ang JE, Rha SY, Yonemori K, Kristeleit R, Lin CC, Satoh T, Garcia PE, Sendur MAN, Rodríguez LM, Italiano A, Lugowska I, Ray-Coquard IL, Oza AM, Zhao JL, Gajavelli S, Filant J, Bodla S, Janjigian YY and Meric-Bernstam F. 714MO datopotamab derux-tecan (Dato-DXd) in patients with endometrial (EC) or ovarian cancer (OC): results from the phase II TROPION-PanTumor03 study. *Ann Oncol* 2024; 35: S547-S548.
- [24] Cheng Y, Yuan X, Tian Q, Huang X, Chen Y, Pu Y, Long H, Xu M, Ji Y, Xie J, Tan Y, Zhao X and Song H. Preclinical profiles of SKB264, a novel anti-TROP2 antibody conjugated to topoisomerase inhibitor, demonstrated promising antitumor efficacy compared to IMMU-132. *Front Oncol* 2022; 12: 951589.
- [25] He N, Yang C, Yang Y, Xue Z, Xu J, Zhao L, Feng J, Ye X, Zhang Z and He F. Abstract LB030: SHR-A1921, a novel TROP-2 ADC with an optimized design and well-balanced profile between efficacy and safety. *Cancer Res* 2023; 83: LB030.
- [26] Kim H, Guerra E, Baek E, Jeong Y, You H, Yu B, Jang T, Saverio A, Chung CW and Park C. LCB84, a TROP2-targeted ADC, for treatment of solid tumors that express TROP-2 using the hu2G10 tumor-selective anti-TROP2 monoclonal antibody, a proprietary site-directed conjugation technology and plasma-stable tumor-selective linker chemistry. *Cancer Res* 2022; 82: 328.
- [27] Cardillo TM, Rossi DL, Zalath MB, Liu D, Arrojo R, Sharkey RM, Chang CH and Goldenberg DM. Predictive biomarkers for sacituzumab govitecan efficacy in Trop-2-expressing triple-negative breast cancer. *Oncotarget* 2020; 11: 3849-3862.
- [28] Zhao M, DiPeri TP, Raso MG, Zheng X, Rizvi YQ, Evans KW, Yang F, Akcakanat A, Roberto Estecio M, Tripathy D, Dumbrava EE, Damodaran S and Meric-Bernstam F. Epigenetically upregulating TROP2 and SLFN11 enhances therapeutic efficacy of TROP2 antibody drug conjugate sacituzumab govitecan. *NPJ Breast Cancer* 2023; 9: 66.
- [29] Lee B, Ji W, Lee JC, Song SY, Shin YS, Cho YH, Park JE, Park H and Choi CM. Efficacy of lazertinib for symptomatic or asymptomatic brain metastases in treatment-naïve patients with advanced EGFR mutation-positive non-small cell lung cancer: protocol of an open-label, single-arm phase II trial. *Thorac Cancer* 2023; 14: 2233-2237.
- [30] Lin NU, Borges V, Anders C, Murthy RK, Paplomata E, Hamilton E, Hurvitz S, Loi S, Okines A, Abramson V, Bedard PL, Oliveira M, Mueller V, Zelnak A, DiGiovanna MP, Bachelot T, Chien AJ, O'Regan R, Wardley A, Conlin A, Cameron D, Carey L, Curigliano G, Gelmon K, Loibl S, Mayor J, McGoldrick S, An X and Winer EP. Intracranial efficacy and survival with tucatinib plus trastuzumab and capecitabine for previously treated HER2-positive breast cancer with brain metastases in the HER2CLIMB trial. *J Clin Oncol* 2020; 38: 2610-2619.
- [31] Nakhjavani M, Samarasinghe RM and Shigdar S. Triple-negative breast cancer brain metastasis: an update on druggable targets, current clinical trials, and future treatment options. *Drug Discov Today* 2022; 27: 1298-1314.
- [32] Jeon Y, Jo U, Hong J, Gong G and Lee HJ. Trophoblast cell-surface antigen 2 (TROP2) expression in triple-negative breast cancer. *BMC Cancer* 2022; 22: 1014.
- [33] Mertens RB, Makhoul EP, Li X and Dadmanesh F. Comparative expression of trophoblast cell-surface antigen 2 (TROP2) in the different molecular subtypes of invasive breast carcinoma: an immunohistochemical study of 94 therapy-naïve primary breast tumors. *Ann Diagn Pathol* 2024; 68: 152226.
- [34] Tanegashima K, Tanaka Y, Ito T, Oda Y and Nakahara T. TROP2 expression and therapeutic implications in cutaneous squamous cell carcinoma: insights from immunohistochemical and functional analysis. *Exp Dermatol* 2024; 33: e15196.