Annals of Oncology 23: 2185–2190, 2012
doi:10.1093/annonc/mdr605
Published online 8 February 2012

High STEAP1 expression is associated with improved outcome of Ewing's sarcoma patients

T. G. P. Grunewald[1,2*,†], A. Ranft[3,†], I. Esposito[4,5,†], P. da Silva-Buttkus[5], M. Aichler[5], D. Baumhoer[6], K. L. Schaefer[7], L. Ottaviano[7], C. Poremba[7,8], G. Jundt[6], H. Jürgens[3], U. Dirksen[3], G. H. S. Richter[1,‡] & S. Burdach[1,‡]

[1]*Children's Cancer Research and Roman Herzog Comprehensive Cancer Center, Laboratory of Functional Genomics and Transplantation Biology, Klinikum rechts der Isar, Technische Universität München, Munich;* [2]*Medical Life Science and Technology Center, TUM Graduate School, Technische Universität München, Garching;* [3]*Department of Pediatric Hematology and Oncology, University Hospital Münster, Münster;* [4]*Institute of Pathology, Klinikum rechts der Isar, Technische Universität München, Munich;* [5]*Institute of Pathology, Helmholtz-Zentrum München, Neuherberg, Germany;* [6]*Bone Tumor Reference Center at the Institute of Pathology, University Hospital Basel, Basel, Switzerland;* [7]*Institute of Pathology, Heinrich-Heine-University, Düsseldorf;* [8]*Center of Histopathology, Cytology, and Molecular Diagnostics (CHCMD), Trier, Germany*

Received 10 August 2011; revised 2 December 2011; accepted 5 December 2011

Background: Ewing's sarcoma (ES) is the second most common bone or soft-tissue sarcoma in childhood and adolescence and features a high propensity to metastasize. The six-transmembrane epithelial antigen of the prostate 1 (STEAP1) is a membrane-bound mesenchymal stem cell marker highly expressed in ES. Here, we investigated the role of STEAP1 as an immunohistological marker for outcome prediction in patients with ES.
Patients and methods: Membranous STEAP1 immunoreactivity was analyzed using immunohistochemistry in 114 primary pre-chemotherapy ES of patients diagnosed from 1983 to 2010 and compared with clinical parameters and patient outcome. Median follow-up was 3.85 years (range 0.43–17.51).
Results: A total of 62.3% of the ES samples displayed detectable STEAP1 expression with predominant localization of the protein at the plasma membrane. High membranous STEAP1 immunoreactivity was found in 53.5%, which correlated with better overall survival ($P = 0.021$). Accordingly, no or low membranous STEAP1 expression was identified as an independent risk factor in multivariate analysis (hazard ratio 2.65, $P = 0.036$).
Conclusion: High membranous STEAP1 expression predicts improved outcome and may help to define a specific subgroup of ES patients, who might benefit from adapted therapy regimens.
Key words: biomarker, Ewing's sarcoma, outcome, risk stratification, STEAP1

introduction

Ewing's sarcoma (ES) is a highly aggressive bone or soft-tissue cancer mostly affecting children and young adolescents [1–4]. Even though multimodal treatments have led to remarkable improvements in survival of patients with localized disease, prognosis of patients with metastatic disease remains poor with an event-free survival of <25% [4–7].

ES is characterized by *EWS–ETS* translocations [8] encoding aberrant transcription factors that determine the complex and highly malignant phenotype of this disease [9]. Although different variants of EWS–ETS fusion proteins exist, they fail to provide reliable biomarkers for individual risk stratification [10, 11]. Several trials proved the clinicopathological parameters, tumor site, tumor volume, age at diagnosis, responsiveness to chemotherapy, and sites of metastatic disease, to have major prognostic value [10, 12, 13]. However, the currently available biological markers for ES are very limited [12, 14, 15]. Nevertheless, the discovery of novel prognostic and/or predictive biomarkers would potentially lead to a better understanding of tumor heterogeneity, enable individual risk stratification, and might help to guide targeted therapy [16–19].

We previously identified an expression signature comprising ~40 genes that are highly overexpressed in ES compared with normal tissues and that might constitute promising candidates for risk prediction and targeted therapy [9, 20]. Among them, we identified the six-transmembrane epithelial antigen of the prostate 1 (STEAP1), which is a membrane-bound channel protein possibly involved in transmembrane electron transfer [21, 22]. Apart from low amounts in prostate and urothelium,

*Correspondence to: Dr T. G. P. Grunewald, Children's Cancer Research and Roman Herzog Comprehensive Cancer Center, Laboratory of Functional Genomics and Transplantation Biology, Klinikum rechts der Isar, Technische Universität München, Kölner Platz 1, 80804 Munich, Germany. Tel: +49-89-3068-5525; Fax: +49-89-3068-3791; E-mail: thomas.gruenewald@lrz.tum.de

†These authors contributed equally to this work.
‡Both authors contributed equally as senior authors.

STEAP1 is virtually not expressed in normal tissues [23, 24]. In contrast, STEAP1 is strongly overexpressed in many cancers including prostate, breast, and bladder carcinoma as well as ES [23–25]. STEAP1 messenger RNA (mRNA) circulates in peripheral blood of cancer patients [26] and its detection in bone marrow is indicative for occult residual tumor cells in patients with ES [27]. Moreover, STEAP1 was found to be a *bona fide* marker for human mesenchymal stem cells [28] lending support to the hypothesis of a mesenchymal origin of ES [29].

In addition, we recently showed that STEAP1 overexpression increases the invasive properties and intracellular levels of reactive oxygen species (ROS) of ES cells [24]. However, the diagnostic potential of STEAP1 for ES remained undetermined.

In the current study, we investigated the value of STEAP1 as an immunohistological marker for outcome prediction of patients with ES. We provide evidence that high membranous STEAP1 expression is associated with improved overall survival (OS). Moreover, high membranous STEAP1 immunoreactivity showed a trend toward a better histological tumor response to chemotherapy and, conversely, STEAP1-silenced ES cells were more resistant to chemotherapy *in vitro*. These data unravel a hitherto unanticipated role of STEAP1 as a promising independent biomarker for outcome prediction of ES.

materials and methods

study population, ES tissue samples, and tissue microarray

The Technische Universität München and the Universities of Basel, Düsseldorf, and Münster approved the current study. A total of 114 archival paraffin-embedded primary ES samples before treatment with confirmed histological diagnosis (reference pathology) were obtained from the Departments of Pathology of the Technische Universität München and the University of Düsseldorf as well as from the Bone Tumor Reference Center at the Institute of Pathology of the University of Basel. Representative formalin-fixed, paraffin-embedded tumor blocks were selected for either tissue microarray (TMA) construction at the Department of Pathology of the University of Düsseldorf (66 samples) or open procedures at the Departments of Pathology of the Technische Universität München (6 samples) and the University of Basel (42 samples). Each TMA slide contained reference tissues of ES xenografts with known STEAP1 expression as internal controls (see supplemental Methods, available at *Annals of Oncology* online).

Pertinent clinical data of patients were compiled from two sources: first, the Ewing trial center of the University Hospital Münster (93 patients enrolled in the CESS 81, CESS 86, EICESS 92, or EURO-E.W.I.N.G. 99 trials) and second, the Department of Pathology of the University of Basel (21 patients). Informed consent was obtained from all patients and/or their legal guardians. The study population included 60 males and 54 females with a median age of 16.9 years (range 0.6–59.8 years).

immunohistochemistry and evaluation of STEAP1 immunoreactivity

Immunohistochemistry (IHC) analyses were done on formalin-fixed, paraffin-embedded, pre-chemotherapy primary tumors. All tissue slides were collected at the Department of Pathology of the Technische Universität München for immediate IHC staining. For IHC, 4-µm sections were cut and stained by an automated immunostainer with an iView DAB detection kit (Ventana Medical System, Tucson, AZ) according to the company's protocol. The following primary antibody was used: polyclonal rabbit anti-STEAP1 (1:50; H-105, sc-25514, Santa Cruz). Antigen retrieval was carried out by microwave treatment in Dako target retrieval solution, citrate, pH 6.0. Sections were counterstained with hematoxylin. For internal controls, we used tumors of xenografted ES cell lines with known STEAP1 mRNA and protein expression levels (see supplemental Methods and Figure S1, available at *Annals of Oncology* online). Specificity of the STEAP1 antibody was assessed previously by others [30, 31] and reassessed by us using immunoblot and indirect immunofluorescence, as previously described [32, 33] (see supplemental Methods and Figure S1, available at *Annals of Oncology* online). These control experiments further confirmed the specificity of the used STEAP1 antibody, in agreement with published findings on the STEAP1 protein [23, 24, 34]. Semi-quantitative evaluation of STEAP1 immunostaining was carried out in a blinded manner by a pathologist (IE) and two scientist experienced in histopathology (PS-B, MA) after having examined at least three high-power fields (40×) of one section for each sample. The intensity of membranous STEAP1 immunoreactivity was determined as grade 0 = none, grade 1 = faint, grade 2 = moderate, and grade 3 = strong (Figure 1). Intensity scoring was independently recorded and in case of disagreement determined by consensus. For better statistical discrimination, samples were classified into two groups as previously described [32, 35]: samples with grade 0 and 1 were classified as STEAP1 low and those with grade 2 and 3 as STEAP1 high.

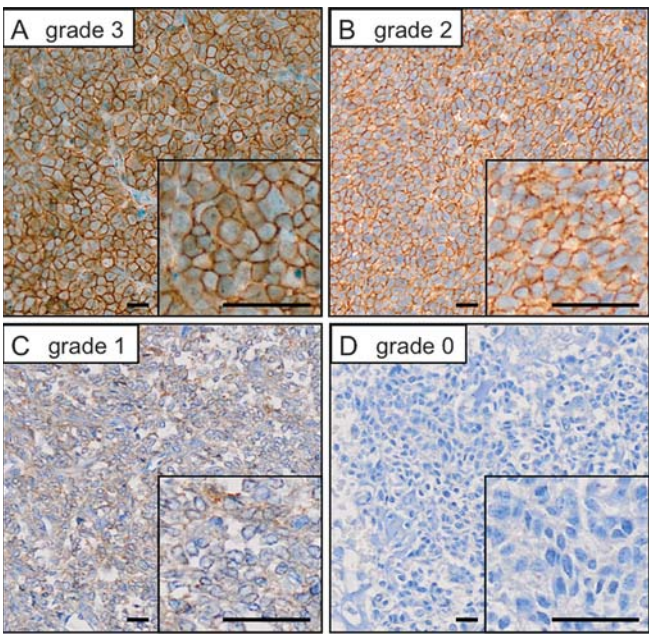

Figure 1. Examples of heterogeneous membranous six-transmembrane epithelial antigen of the prostate 1 (STEAP1) immunoreactivity in Ewing's sarcoma (ES): All samples depicted were located on the same tissue microarray slide and stained simultaneously by an automated immunostainer (see 'materials and methods' section). Membranous STEAP1 immunoreactivity (brown color) was scored according to reference ES with known STEAP1 expression levels, with grade 3 = strong (A), grade 2 = moderate (B), grade 1 = faint (C), and grade 0 = no immunoreactivity (D). Grades 3 and 2 were classified as STEAP1 high and grades 1 and 0 as STEAP1 low. Scale bars = 20 µm for overview and 80 µm for detail images.

statistical analyses

Statistical analyses were carried out with SPSS 19 (IBM Corporation, Armonk, NY) and SAS 9.2 (SAS Institute, Cary, NC). OS was estimated by the Kaplan–Meier method. OS time was defined as the interval between the date of diagnosis and the date of last follow-up or death. Living patients were censored at the date of most recent consultation. Group comparisons were calculated by log-rank test. Multivariate analyses were carried out by applying the Cox proportional hazard method. Differences in proportions between groups were evaluated by chi-square or Fisher's exact test. Significance level was set at $P < 0.05$ for two-sided testing. No alpha corrections were carried out for multiple testing. Outcome was analyzed on an exploratory basis.

results

STEAP1 is expressed in the majority of ES and mainly locates to plasma membranes

We first aimed to define the expression pattern of STEAP1 in ES. Of the 114 ES available for IHC, 71 displayed detectable membranous STEAP1 immunoreactivity (62.3%, grades 1–3). Examples of the differential membranous STEAP1 immunoreactivity are given in Figure 1. A total of 53.5% ($n = 61$) of the ES were scored as membranous STEAP1 high and 46.5% ($n = 53$) as membranous STEAP1 low; 24.6% (28 of 114) of the cases showed maximum membranous STEAP1 expression (grade 3; see Figure 1). In agreement with previous findings in breast, bladder, and prostate carcinoma [21, 23, 25], we noted a predominant plasma membranous localization of STEAP1 without defined apical or basal accentuation and mostly without cytoplasmic STEAP1 immunoreactivity. Only a few ES samples showed a faint to moderate cytoplasmic STEAP1 staining.

membranous STEAP1 expression and OS

We next aimed to determine whether membranous STEAP1 expression correlates with outcome of ES patients. Patient characteristics are given in Table 1. Univariate analysis on the predictive value of membranous STEAP1 immunoreactivity showed a lower survival rate in patients with ES classified as membranous STEAP1-low (5-year OS = 0.57; $n = 53$) when compared with membranous STEAP1-high cases (5-year OS = 0.79; $n = 61$) ($P = 0.021$) (Figure 2).

multivariate analysis

We next analyzed the impact of risk stratification in patients with membranous STEAP1-high ES compared with patients with membranous STEAP1-low ES to rule out a possible bias by favorable risk factor patterns in STEAP1-high cases. The multivariate analysis served to identify underlying factors that could influence prognosis. We included the known prognostic factors metastatic stage at diagnosis (M0, M1, M2), site (axial versus nonaxial), and age (<15 versus ≥15 years) [7, 12, 13] in the multivariate analysis. Eighty-three patients (72.8%) had localized disease (M0), 20 patients (17.5%) had pulmonary metastases (M1), and 11 patients (9.6%) had disseminated disease including other metastases than in lungs (M2). Sixty-six patients (57.9%) presented with an axial site ES and 48 patients (42.1%) with a non-axial site ES. Forty-six patients (40.4%) were aged <15 years, and 68 patients (59.6%) were aged >15 years at time of diagnosis.

Table 1. Patient characteristics ($n = 114$)

Variable	Label	*n* (%)
Sex	Male	60 (52.6)
	Female	54 (47.4)
Age at diagnosis	<15 years	46 (40.4)
	≥15 years	68 (59.6)
Risk group	M0 (no metastases)	83 (72.8)
	M1 (lung metastases)	20 (17.5)
	M2 (other ± lung metastases)	11 (9.7)
Site	Axial	66 (57.9)
	Non-axial	48 (42.1)
Tumor volume[a]	<200 ml	55 (71.4)
	≥200 ml	22 (28.6)
Histological response[a]	Good (<10% viable cells)	46 (78.0)
	Poor (≥10% viable cells)	13 (22.0)
Membranous six-transmembrane epithelial antigen of the prostate 1 expression	How	53 (46.5)
	High	61 (53.5)

[a]These parameters relate to subsets of the study population.

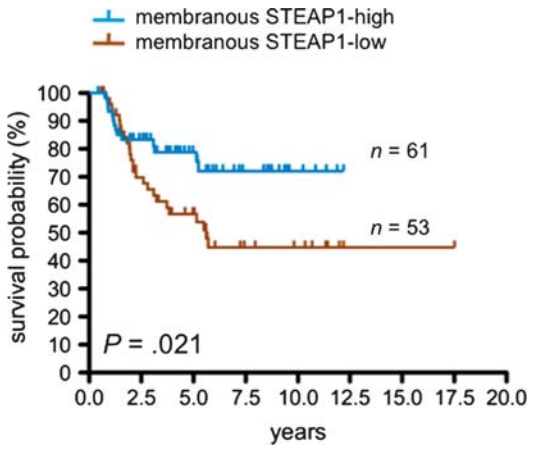

Figure 2. Six-transmembrane epithelial antigen of the prostate 1 (STEAP1) expression correlates with overall survival (OS): Kaplan–Meier estimates for OS probability for membranous STEAP1 expression ($n = 114$, $P = 0.021$). Log-rank test.

The major risk factor was metastatic disease at diagnosis [M0: hazard ratio (HR) = 1.00; M1: HR = 2.19; M2: HR = 4.38; $P = 0.002$]. Membranous STEAP1-low expression (HR = 1.76; $P = 0.094$), age (>15 years, HR = 1.69; $P = 0.135$), and primary axial tumor site (HR 1.30; $P = 0.435$) showed only a tendency or no major impact on survival ($n = 114$; Table 2).

In a second step, we analyzed the major group of patients with localized disease (M0; $n = 83$). Here, membranous STEAP1-low expression (HR = 2.59; $P = 0.036$) and age (>15 years; HR = 3.39; $P = 0.030$) were major risk factors concerning survival in relation to primary axial tumor site (HR 1.76; $P = 0.218$) (Table 3), which most likely still only showed a

Table 2. Summary of results of the multivariate analysis in all patients ($n = 114$)

Variable	Label	Risk ratio (95% CI)	P
Risk group	M0 (no metastases)	1	0.002
	M1 (lung metastases)	2.19 (1.04–4.61)	0.039
	M2 (other ± lung metastases)	4.38 (1.83–10.5)	0.001
Membranous STEAP1 expression	Low	1.78 (0.91–3.48)	0.094
Age	≥15 years	1.69 (0.85–3.37)	0.135
Site	Axial	1.30 (0.68–2.48)	0.435

STEAP1, six-transmembrane epithelial antigen of the prostate 1 and CI, confidence interval.

Table 3. Summary of results of the multivariate analysis in patients with localized disease ($n = 83$)

Variable	Label	Risk ratio (95% CI)	P
Membranous STEAP1 expression	Low	2.59 (1.07–6.29)	0.036
Age	≥15 years	3.39 (1.13–10.2)	0.030
Site	Axial	1.76 (0.72–4.31)	0.218

STEAP1, six-transmembrane epithelial antigen of the prostate 1 and CI, confidence interval.

tendency on survival due to the limited size of our patient cohort.

Tumor volume: A tumor volume categorization (<200 versus ≥200 ml) was only available in 77 patients. Fifty-five patients (71.4%) had a tumor volume <200 ml and 22 patients (28.6%) had a tumor volume >200 ml. Multivariate analysis in this subcohort of 77 patients adding tumor volume as known prognostic factor to the other established prognostic factors described above (metastatic disease at diagnosis, age >15 years, and primary axial tumor site [7, 12, 13]) confirmed metastatic disease at diagnosis ($P = 0.002$) and membranous STEAP1-low expression (HR = 2.65; $P = 0.036$) as major risk factors.

association of STEAP1 expression with histological response to chemotherapy

Since high membranous STEAP1 immunoreactivity correlated with improved OS, we tested whether this observation is associated with a better response to treatment as estimated by Salzer–Kuntschik tumor regression states [36]. For 59 patients (51.8%), data were available for histological response following induction chemotherapy without concurrent early radiotherapy. Forty-six patients (78%) showed a good histological response (<10% viable tumor cells) and 13 patients (22%) a poor histological response (≥10% viable tumor cells); 80.6% of the patients with membranous STEAP1-high expression showed a good histological response compared with 73.9% of the patients with membranous STEAP1-low expression ($P = 0.748$) (Table 4). To test whether differential STEAP1 expression may indeed alter response to chemotherapy *in vitro*, we transiently knocked down STEAP1 in cultured ES cells by RNA interference and assessed their rates of necrosis by flow cytometry. STEAP1-silenced ES cells treated with either doxorubicin or etoposide for 24 h exhibited lower rates of necrosis compared with ES cells with high STEAP1 expression ($P < 0.01$; t-test, $n = 4$) (Figure S2), indicating that low STEAP1 expression may confer ES cells with a more resistant phenotype to chemotherapy.

Table 4. Summary of results of correlation of membranous STEAP1 immunoreactivity with tumor regression grade ($n = 59$)

		Histological response		
		Good	Poor	P
Membranous STEAP1 expression	Low	17 (73.9%)	6 (26.1%)	
	High	29 (80.6%)	7 (19.4%)	0.748

STEAP1, six-transmembrane epithelial antigen of the prostate 1.

discussion

Combined modality treatment is crucial for successful therapy of patients with ES [7]. So far, various studies have identified metastatic state, tumor volume, tumor site, age, sex, and histological response to chemotherapy as important risk factors, with primary metastasis as the most unfavorable one [7, 12, 13, 37]. Although there is agreement that clinical management will benefit from biological markers that can guide therapeutic decisions [12], apart from the proliferation marker Ki67 [38, 39], there are no *bona fide* immunohistological markers available predicting outcome of patients with ES [12, 15].

This is, to the best of our knowledge, the first report evaluating the potential of STEAP1 for outcome prediction in ES. Regarding independent risk factors in our series, high membranous STEAP1 expression had a strong impact on OS in multivariate analysis.

Moreover, membranous STEAP1-high immunoreactivity showed a trend toward a better tumor response compared with membranous STEAP1-low immunoreactivity as estimated by Salzer–Kuntschik regression states. Even though this subsample tendency has to be validated in a larger cohort, it is noteworthy that high STEAP1 expression improves response of ES cells to chemotherapy *in vitro*. Hence, it is tempting to speculate that STEAP1 may exert a biological function that sensitizes ES to drugs such as doxorubicin and etoposide, which are essential components of current ES treatment protocols [40].

On the molecular level, STEAP1 is a homologue of NADPH oxidases [41, 42], which are involved in cellular ROS production and frequently overexpressed in cancer [43, 44]. Consistent with this notion, we recently demonstrated that STEAP1 overexpression in ES cells increases their intracellular ROS levels [24]. Similar observations were obtained by Pan et al. in thyroid epithelial cells [45] Pharmacologically, multiple studies demonstrated that certain chemotherapeutics like

doxorubicin and etoposide become more potent at increased intracellular ROS levels [46, 47]. Moreover, radiotherapy is known to be more effective in combination with ROS-generating radiosensitizers [47, 48]. Thus, the apparent survival benefit seen in membranous STEAP1-high ES patients may be caused by elevated intracellular ROS levels of the ES cells, which might sensitize them to radiochemotherapy.

As outlined above, metastasis of ES is the most adverse clinical parameter indicative for dismal prognosis with a 5-year relapse-free survival of ~21% compared with 55% in patients with localized disease. Strikingly, the observed survival benefit of membranous STEAP1-high compared with membranous STEAP1-low immunoreactivity is similarly strong like the dramatic difference in survival indicated by localized versus metastatic disease. Hence, our data suggest that high membranous STEAP1 expression may be a property of an independent risk group of ES patients, who specifically might benefit from adapted radio-chemotherapy protocols.

Despite we are fully aware of the retrospective nature of this study and its associated limitations, STEAP1 may constitute a promising new biomarker for outcome prediction of ES patients, which is readily available due to standardized assessment by immunohistochemistry. Therefore, we strongly recommend to validate this observation in prospective studies and to experimentally elucidate the precise molecular role of STEAP1 in ES.

acknowledgements

We gratefully acknowledge the contribution of the participating Gesellschaft für Pädiatrische Onkologie und Hämatologie institutions and of the staff of the Ewing trial center Münster, Susanne Jabar and Regina Kloss. We thank Barbara Grunewald, Uwe Thiel, Stephanie Plehm, and Veit R. Buchholz for critical reading of the manuscript.

funding

Technische Universität München (KKF B08-05 to T.G. and A09-02 to G.R.); Dr. Sepp und Hanne Sturm Gedächtnisstiftung of the city of Munich (to T.G., G.R., and S. B.); TUM Graduate School (to T.G.); Deutsche Forschungsgemeinschaft (DFG GR3728/1-1 to T.G., G.R., and S.B.); Wilhelm-Sander-Stiftung (2009.901.1 to G.R. and S.B.); Deutsche Krebshilfe (50-2551-Jü3 and 50-2551-Jü4 to H.J.); BMBF (FK 01GM0870) to Translational Sarcoma Research Network; National Genome Network (NGFNplus) to IE's laboratory.

disclosure

The authors declare no conflict of interest.

references

1. Burdach S, Thiel U, Schöniger M et al. Total body MRI-governed involved compartment irradiation combined with high-dose chemotherapy and stem cell rescue improves long-term survival in Ewing tumor patients with multiple primary bone metastases. Bone Marrow Transplant 2010; 45(3): 483–489.
2. Burdach S. Treatment of advanced Ewing tumors by combined radiochemotherapy and engineered cellular transplants. Pediatr Transplant 2004; 8 (Suppl 5): 67–82.
3. Burdach S, Jurgens H. High-dose chemoradiotherapy (HDC) in the Ewing family of tumors (EFT). Crit Rev Oncol Hematol 2002; 41(2): 169–189.
4. Thiel U, Wawer A, Wolf P et al. No improvement of survival with reduced- versus high-intensity conditioning for allogeneic stem cell transplants in Ewing tumor patients. Ann Oncol 2011; 22(7): 1614–1621.
5. Burdach S, Jurgens H, Peters C et al. Myeloablative radiochemotherapy and hematopoietic stem-cell rescue in poor-prognosis Ewing's sarcoma. J Clin Oncol 1993; 11(8): 1482–1488.
6. Burdach S, Meyer-Bahlburg A, Laws HJ et al. High-dose therapy for patients with primary multifocal and early relapsed Ewing's tumors: results of two consecutive regimens assessing the role of total-body irradiation. J Clin Oncol 2003; 21(16): 3072–3078.
7. Haeusler J, Ranft A, Boelling T et al. The value of local treatment in patients with primary, disseminated, multifocal Ewing sarcoma (PDMES). Cancer 2010; 116(2): 443–450.
8. Delattre O, Zucman J, Melot T et al. The Ewing family of tumors—a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 1994; 331(5): 294–299.
9. Staege MS, Hutter C, Neumann I et al. DNA microarrays reveal relationship of Ewing family tumors to both endothelial and fetal neural crest-derived cells and define novel targets. Cancer Res 2004; 64(22): 8213–8221.
10. Le Deley M-C, Delattre O, Schaefer K-L et al. Impact of EWS-ETS fusion type on disease progression in Ewing's sarcoma/peripheral primitive neuroectodermal tumor: prospective results from the cooperative Euro-E.W.I.N.G. 99 trial. J Clin Oncol 2010; 28(12): 1982–1988.
11. Ginsberg JP, de Alava E, Ladanyi M et al. EWS-FLI1 and EWS-ERG gene fusions are associated with similar clinical phenotypes in Ewing's sarcoma. J Clin Oncol 1999; 17(6): 1809–1814.
12. Riley RD, Burchill SA, Abrams KR et al. A systematic review and evaluation of the use of tumour markers in paediatric oncology: Ewing's sarcoma and neuroblastoma. Health Technol Assess 2003; 7(5): 1–162.
13. Cotterill SJ, Ahrens S, Paulussen M et al. Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. J Clin Oncol 2000; 18(17): 3108–3114.
14. Bui MM, Han G, Acs G et al. Connexin 43 is a potential prognostic biomarker for Ewing sarcoma/primitive neuroectodermal tumor. Sarcoma 2011; 2011: 971050.
15. de Alava E, Antonescu CR, Panizo A et al. Prognostic impact of P53 status in Ewing sarcoma. Cancer 2000; 89(4): 783–792.
16. Lee CK, Lord SJ, Coates AS, Simes RJ. Molecular biomarkers to individualise treatment: assessing the evidence. Med J Aust 2009; 190(11): 631–636.
17. Nishio K, Arao T, Shimoyama T et al. Translational studies for target-based drugs. Cancer Chemother Pharmacol 2005; 56(Suppl 1): 90–93.
18. Grunewald TG, Herbst SM, Heinze J, Burdach S. Understanding tumor heterogeneity as functional compartments—superorganisms revisited. J Transl Med 2011; 9(1): 79.
19. Juergens H, Daw NC, Geoerger B et al. Preliminary efficacy of the anti-insulin-like growth factor type 1 receptor antibody figitumumab in patients with refractory Ewing sarcoma. J Clin Oncol 2011 Dec 1; 29(34): 4534–40. Epub 2011 Oct 24.
20. Thiel U, Pirson S, Müller-Spahn C et al. Specific recognition and inhibition of Ewing tumour growth by antigen-specific allo-restricted cytotoxic T cells. Br J Cancer 2011; 104(6): 948–956.
21. Challita-Eid PM, Morrison K, Etessami S et al. Monoclonal antibodies to six-transmembrane epithelial antigen of the prostate-1 inhibit intercellular communication in vitro and growth of human tumor xenografts in vivo. Cancer Res 2007; 67(12): 5798–5805.
22. Ohgami RS, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. Blood 2006; 108(4): 1388–1394.

23. Hubert RS, Vivanco I, Chen E et al. STEAP: a prostate-specific cell-surface antigen highly expressed in human prostate tumors. Proc Natl Acad Sci U S A 1999; 96(25): 14523–14528.
24. Grunewald TG, Diebold I, Esposito I et al. STEAP1 is associated with the invasive and oxidative stress phenotype of Ewing tumors. Mol Cancer Res 2012; 10(1): 52–65.
25. Maia CJ, Socorro S, Schmitt F, Santos CR. STEAP1 is over-expressed in breast cancer and down-regulated by 17beta-estradiol in MCF-7 cells and in the rat mammary gland. Endocrine 2008; 34(1–3): 108–116.
26. Valenti MT, Dalle Carbonare L, Donatelli L et al. STEAP mRNA detection in serum of patients with solid tumours. Cancer Lett 2009; 273(1): 122–126.
27. Cheung IY, Feng Y, Danis K et al. Novel markers of subclinical disease for Ewing family tumors from gene expression profiling. Clin Cancer Res 2007; 13(23): 6978–6983.
28. Vaghjiani RJ, Talma S, Murphy CL. Six-transmembrane epithelial antigen of the prostate (STEAP1 and STEAP2)-differentially expressed by murine and human mesenchymal stem cells. Tissue Eng Part A 2009; 15(8): 2073–2083.
29. Tirode F, Laud-Duval K, Prieur A et al. Mesenchymal stem cell features of Ewing tumors. Cancer Cell 2007; 11(5): 421–429.
30. Klein A, Guhl E, Zollinger R et al. Gene expression profiling: cell cycle deregulation and aneuploidy do not cause breast cancer formation in WAP-SVT/t transgenic animals. J Mol Med (Berl) 2005; 83(5): 362–376.
31. Kobayashi H, Nagato T, Sato K et al. Recognition of prostate and melanoma tumor cells by six-transmembrane epithelial antigen of prostate-specific helper T lymphocytes in a human leukocyte antigen class II-restricted manner. Cancer Res 2007; 67(11): 5498–5504.
32. Grunewald TGP, Kammerer U, Kapp M et al. Nuclear localization and cytosolic overexpression of LASP-1 correlates with tumor size and nodal-positivity of human breast carcinoma. BMC Cancer 2007; 7: 198.
33. Grunewald TGP, Kammerer U, Winkler C et al. Overexpression of LASP-1 mediates migration and proliferation of human ovarian cancer cells and influences zyxin localisation. Br J Cancer 2007; 96(2): 296–305.
34. Hayashi S, Kumai T, Matsuda Y et al. Six-transmembrane epithelial antigen of the prostate and enhancer of zeste homolog 2 as immunotherapeutic targets for lung cancer. J Transl Med 2011; 9: 191.
35. Frietsch JJ, Grunewald TGP, Jasper S et al. Nuclear localisation of LASP-1 correlates with poor long-term survival in female breast cancer. Br J Cancer 2010; 102(11): 1645–1653.
36. Salzer-Kuntschik M, Brand G, Delling G. [Determination of the degree of morphological regression following chemotherapy in malignant bone tumors]. Pathologe 1983; 4(3): 135–141.
37. Rodríguez-Galindo C, Liu T, Krasin MJ et al. Analysis of prognostic factors in Ewing sarcoma family of tumors: review of St. Jude Children's Research Hospital studies. Cancer 2007; 110(2): 375–384.
38. López-Guerrero JA, Machado I, Scotlandi K et al. Clinicopathological significance of cell cycle regulation markers in a large series of genetically confirmed Ewing's sarcoma family of tumors. Int J Cancer 2011; 128(5): 1139–1150.
39. Mackintosh C, Ordóñez JL, García-Domínguez DJ et al. 1q gain and CDT2 overexpression underlie an aggressive and highly proliferative form of Ewing sarcoma. Oncogene 2011 Aug 8 [Epub ahead of print] .
40. Juergens C, Weston C, Lewis I et al. Safety assessment of intensive induction with vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) in the treatment of Ewing tumors in the EURO-E.W.I.N.G. 99 clinical trial. Pediatr Blood Cancer 2006; 47(1): 22–29.
41. Sanchez-Pulido L, Rojas AM, Valencia A et al. ACRATA: a novel electron transfer domain associated to apoptosis and cancer. BMC Cancer 2004; 4: 98.
42. von Rozycki T, Yen MR, Lende EE, Saier MH. The YedZ family: possible heme binding proteins that can be fused to transporters and electron carriers. J Mol Microbiol Biotechnol 2004; 8(3): 129–140.
43. Kamata T. Roles of Nox1 and other Nox isoforms in cancer development. Cancer Sci 2009; 100(8): 1382–1388.
44. Lambeth JD. NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 2004; 4(3): 181–189.
45. Pan YZ, Li Y, Guo LR et al. [Influence of expression of six transmembrane epithelial antigen of the prostate-1 on intracellular reactive oxygen species level and cell growth: an in vitro experiment]. Zhonghua Yi Xue Za Zhi 2008; 88(9): 641–644.
46. Mailloux RJ, Adjeitey CN, Harper ME. Genipin-induced inhibition of uncoupling protein-2 sensitizes drug-resistant cancer cells to cytotoxic agents. PLoS One 2010; 5(10): e13289.
47. Sun Y, St Clair DK, Xu Y et al. A NADPH oxidase-dependent redox signaling pathway mediates the selective radiosensitization effect of parthenolide in prostate cancer cells. Cancer Res 2010; 70(7): 2880–2890.
48. Girdhani S, Bhosle SM, Thulsidas SA et al. Potential of radiosensitizing agents in cancer chemo-radiotherapy. J Cancer Res Ther 2005; 1(3): 129–131.