# Genomic features of mediastinal germ cell tumors: a narrative review

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**Background and Objective:** Germ cell tumors (GCTs) are uncommon malignancies generally originating from gonads. However, about 5% of GCTs arise outside the gonad (extragonadal), of which 80% develop from the mediastinum. While the prognosis of seminomas is not affected by the gonadal or extragonadal primary location, the prognosis of nonseminoma primary mediastinal GCTs (NS-PMGCTs) is poor, compared to its gonadal counterpart with an estimated 5-year overall survival of about 50%. The current treatments are sub-optimal to increase the cure rate of these rare GCTs. Therefore, molecular insights into these tumors would be valuable to develop novel therapies. The main objective of this review is to describe and dissect the genomic features associated with primary mediastinal GCTs (PMGCTs), highlighting the more frequent genomic alterations and their correlation with clinical outcomes.

**Methods:** We conducted a narrative review of the English literature available in PubMed and Google Scholar between 1982 and 2021, including meta-analyses, systematic reviews, case series and case reports regarding the genomic and clinical features of PMGCTs. We analyzed the available data to describe the molecular characteristics of PMGCTs compared to testicular GCTs (TGCTs), highlighting the most relevant biological and prognostic factors.

**Key Content and Findings:** The high percentage of platinum resistance, the unique association with hematologic malignancies (HMs) and other malignancies, the higher prevalence of *P53* mutations, and a distinct genomic landscape characterize this rare disease.

**Conclusions:** Although some studies have unveiled recurrent molecular alterations in PMGCTs, few are particularly suitable for targeted therapy. Due to the rarity of PMGCTs, data sharing and the creation of an international consortium would be helpful to have a better understanding of the molecular drivers of these tumors.

**Keywords:** Primary mediastinal germ cell tumors (PMGCTs); prognostic biomarkers; poor prognosis; genomic alterations

## Introduction

Testicular cancer is the most prevalent solid tumor in young men aged between 15 and 45 years. Generally, testicular cancer accounts for approximately 1% of all male tumors, and Germ cell tumors (GCTs) make up 95% of tumors arising from testes (1,2). Seminoma and nonseminoma are the main histologic subtypes of GCTs, with nonseminoma being more aggressive than seminoma (3). Around 5% of

**Table 1** The search strategy summary

| Items | Specification |
|---|---|
| Date of search | 2021/10/30 to 2021/11/7 |
| Databases and other sources searched | PubMed and Google Scholar |
| Timeframe | 1982–2021 |
| Inclusion criteria | Meta-analyses, systematic reviews, case series and case reports regarding the genomic and clinical features of PMGCTs published in English |
| Exclusion criteria | Papers which we considered with low reliability and non-English papers |
| Selection Process | The search conducted by Guliz Ozgun and the paper selection was made after discussion with the corresponding author Lucia Nappi |

PMGCTs, primary mediastinal germ cell tumors.

GCTs originate from extragonadal sites, mainly from the anterior mediastinum, followed by the retroperitoneum, pineal gland, and suprasellar regions and the most common histologic subtype of primary mediastinal GCTs (PMGCTs) is nonseminoma (70–80%) (4,5). Mediastinal seminomas are slow-growing tumors, but they can be bulky by the time they are diagnosed due to their indolent course (6). Most seminoma cases have elevated levels of beta human chorionic gonadotropin (hCG) and lactate dehydrogenase (LDH) at the time of diagnosis. Conversely, non-teratoma nonseminoma (NS)-PMGCTs are aggressive tumors with a poor prognosis and an estimated 5-year overall survival rate of around 40–50%. Aside from seminomas, they also have elevated levels of alfa-fetoprotein (AFP) which always shows the existence of a nonseminoma component. Poor prognosis is mainly related to the lower sensitivity of these tumors to cisplatin-based chemotherapy and the limited success of salvage second-line therapies, including high-dose chemotherapy with autologous bone marrow transplant that has not majorly affected the survival of NS-PMGCTs (7).

Better approaches to treat these patients are needed. There have been small-scale clinical researches about PMGCTs so far, which could not generate reliable information. Thanks to The Genome Cancer Atlas (TGCA) project, the molecular background responsible for cancer development provided crucial insights about disease and translational potential for rare diseases. Multiomics data (genome, proteome, transcriptome, epigenome, microbiome) may provide actionable data for biomarkers discovery to better classify the patients and to create a personalized treatment approach for each patient. For PMGCTs, the mechanism of lower sensitivity to chemotherapy remains unclear. However, recent data have demonstrated a higher prevalence of *TP53* mutations in cisplatin-resistant GCTs and NS-PMGCTs, suggesting loss of P53 function as a possible mechanism responsible for the poor response of the disease to treatments (8,9). There might be other possible mechanisms to explore for a better understanding of this rare disease.

Multi-omics studies in GCTs are scarce and are even more limited in PMGCTs, for which more studies are warranted. This review summarizes the available data describing the molecular characteristics of PMGCTs compared to testicular GCTs (TGCT), highlighting the most relevant prognostic factors and possibly uncovering some molecular pathways which could be helpful for future patients' treatment selection and implementation. We present the following article in accordance with the Narrative Review reporting checklist (available at https://med.amegroups.com/article/view/10.21037/med-22-4/rc).

## Methods

Relevant studies published between 1982 and 2021 were identified via a PubMed and Google Scholar search using different combinations of the following terms: "mediastinal germ cell tumors (MGCTs)", "prognostic biomarkers", "genomic alterations", "clinical features", "prognostic factors". Additional papers were identified by reviewing reference lists of relevant publications. Publications with relatively low reliability and which were not written in English were excluded. Data were extracted based on their relevance to the topic. More details of the method are shown in *Table 1*.

## PMGCTs

While the incidence of primary gonadal GCTs has been consistently rising in the last three decades, extragonadal GCT incidence has remained steady overtime (10). PMGCTs account for 1–3% of all germ cell malignancies and for 15% of adult anterior mediastinal cancers. Thymoma, thymic carcinoma, teratoma, thyroid goiter, sarcomas, and lymphomas are considered in the differential diagnosis of anterior mediastinal masses. High levels of LDH, AFP, and/or beta hCG are a meaningful tools to suspect PMGCTs. While AFP is mostly positive in embryonal carcinoma, teratoma and yolk sac tumors, higher beta hCG levels are detected in seminomas, choriocarcinomas, and embryonal carcinomas. Other immune markers such as placental alkaline phosphatase (PLAP) (can be detected in yolk sac tumors and seminoma), leukocyte common antigen (LCA) (lymphoma), desmin/vimentin (sarcomas) and CD30 (embryonal carcinoma) can be helpful in the differential diagnosis, especially by immunohistochemistry and in challenging cases for the diagnosis.

The most accredited mechanism of extragonadal GCTs pathogenesis consists of a defective migration of primordial germ cells along the urogenital ridge or reverse migration of germ cells from testes following the nerve roots (11,12).

Seminomas are usually diagnosed at an older age than nonseminoma testicular GCTs (NS-TGCTs) (median age at diagnosis 34 *vs.* 30, P=0.02), and cryptorchidism is more common in patients with seminoma (P=0.008) (13). Isochromosome 12p [i(12p)] is the widespread shared cytogenetic abnormality in gonadal and mediastinal GCTs and is equally represented in seminomas and nonseminomas (4,14). Seminomas are highly sensitive to cisplatin-based chemotherapy and radiotherapy (RT) and this sensitivity is not affected by the primary tumor location. Therefore, seminoma PMGCT (S-PMGCT) and seminoma TGCT (S-TGCT) share the same prognosis established by the International Germ Cell Consensus Classification (IGCCC) (15).

NS-PMGCTs include teratoma, yolk sac tumor, choriocarcinoma, and/or embryonal carcinoma. Pure or mixed yolk sac tumors are the most common NS-PMGCTs subtypes, while embryonal carcinoma is the less frequent. The prognosis of pure mature primary mediastinal teratoma is excellent and these tumors are usually successfully managed with surgery. However, non-teratoma NS-PMGCTs have a poorer prognosis than their gonadal counterpart. Although PMGCTs and primary gonadal testis cancer share common histologic and clinical characteristics, PMGCTs have distinct features, including association with Klinefelter's syndrome, hematologic malignancies (HMs), and distinct molecular features (16-19).

## Molecular features of GCT precursors

Progenitor germ cells (PGCs) express pluripotency markers such as OCT3/4 and NANOG and have low DNA methylation levels. As these cells are essential for normal germline development, delay or block of the PGCs maturation steps can trigger the development of germ cell neoplasia in situ (GCNIS) (20). These lesions are precursors of invasive malignant GCTs. The presence of i(12p) in GCNIS is associated with a faster progression to invasive GCT. However, how exactly the amplification of the genes located in the chromosome 12p participates in the pathogenesis of GCTs remains unclear (21).

Seminomas resemble PGC and GCNIS in terms of histology, methylation, and gene expression features. The common precursor of nonseminoma GCTs (NSGCTs) is embryonal carcinoma, originating from epigenetic reprogramming of GCNIS or seminomas. As embryonal carcinomas are characterized by pluripotency of embryonic stem cells, they can differentiate in yolk sac tumors, choriocarcinoma, and teratoma (20,22).

Although chromosomal abnormalities are commonly seen in GCNIS and GCTs, somatic genomic mutations are infrequent, especially in early GCNIS, and they slightly increase with the patient's age. Therefore, most somatic TGCT mutations are passenger mutations that accumulate with the patient's age, usually after the early onset of the disease (23).

## Genomic landscape of TGCT and PMGCT

### Genomics of TGCTs

TGCTs are characterized by a very low tumor mutational burden of approximately 0.5 mutations per Mb (0.50 in seminoma and 0.49 in nonseminoma), the second-lowest mutational burden in solid malignancies after Ewing sarcoma (0.3/Mb). TGCTs have high CG>TA transitions [27% of single-nucleotide variants (SNVs)], TA>CG transitions (23%) and CG>AT transversions (31%) (13,23,24).

Copy number alterations (CNAs) have been described in 47% of the nonseminoma patients and are mainly associated with high tumor aneuploidy, which is however

not well established in seminoma patients (25). This high prevalence of aneuploidy is most likely induced by tetraploidization followed by chromosome loss. The most common chromosome change in TGCTs is gain of chromosome 12p, observed in 80% of cases, which is considered pathognomonic of TGCT. Studies mapping the 12p region have failed to identify the central TGCT driver genes. Other chromosome changes, including chromosomes 7, 8, 21, 22, and X gain and loss of chromosome Y, have been described in 25–40% of TGCTs patients. Seminomas have less frequent copies of 11q, whereas nonseminomas have fewer copies of chromosomes 8, 9, 15, 19, 22. Like TGCTs, PMGCTs are mostly aneuploid with large-scale CNAs, including chromosome 12p and X amplification and deletion of the Y chromosome (26-28).

The fibrous sheath interacting protein 2 (*FSIP2*), located in 2q32.1, is the most frequently copy number altered gene in TGCTs, and its focal amplification has been observed in >15% of cases. Interestingly, dysplasia of the fibrous sheath and mutations in *AKAP4* (which binds to FSIP2) have been associated with male infertility, a well-known risk factor for TGCT (23,29).

Overall, 44 risk loci for TGCT have been described in genome-wide association studies (GWAS). The most frequent risk locus for TGCT is 12q21, including the *KITLG* gene that codifies for the c-KIT ligand. This receptor and its substrate have a crucial role in regulating germ cells, and the c-KIT pathway is a central driver in TGCT (30). *c-KIT* mutations occur mainly in exon 17 and rarely in exon 11, and they are more frequent in seminoma than in nonseminoma (18–25% of cases *vs.* 2%, respectively) (31,32). In fact, seminoma and NSGCTs present slightly different genomic alterations. Other than *c-KIT*, the most frequent gene alterations described in S-TGCTs are *KRAS* (14%) and *NRAS* (4%) mutations. In NSGCTs, *c-KIT* and *KRAS* mutations are reported only in a small proportion of patients. Moreover, while seminomas are usually hypertriploid, nonseminomas have lower ploidy and more frequent i(12p) than seminomas. Overall, tumor mutational burden is comparable in seminomas and NSGCTs (13,33,34).

Compared with *c-KIT*-mutated seminomas, *c-KIT*-wild-type seminomas exhibited unique features, including significant lymphocyte infiltration, lack of global DNA methylation, decreased CNAs, i(12p) gain, and a more frequent association with cryptorchidism. Moreover, *c-KIT*-mutated seminomas have a lower rate of *KRAS* mutations, and conversely, *c-KIT* wild-type seminomas have

increased copies of *KRAS* (P=0.0007) (13,35). However, the biological relevance of these alterations is unclear, and despite the high prevalence of activating *c-KIT* mutations and overexpression, clinical trials using imatinib, a c-KIT inhibitor, in platinum-resistant GCT patients failed to demonstrate relevant activity (36).

### Epigenetics of TGCTs

DNA methylation patterns differ among histology subtypes of GCTs. While embryonal carcinoma demonstrates a widespread methylation pattern at non-canonical cytosine sites (e.g., CpA, CpT, CpC), known as CpH sites, other NSGCT subtypes (teratoma, yolk sac, or mixed GCTs) have a stroma-like methylation profile (soma and extra-embryonal lineages) (37,38). DNA methyltransferase is overexpressed in NSGCTs, and preclinical and early phase I studies have demonstrated that their inhibition increases cisplatin activity in cisplatin refractory TGCT cells (39). Methylation is associated with epigenetic silencing of tumor suppressors such as *BRCA1*, *RAD51C*, *MGMT*, and *RASSF1A* in NSGCTs. Thirty-five percent of non-embryonal carcinoma NSGCT cases have *BRCA1* and *RAD51C* promoter methylation, suggesting a possible role for PARP inhibitors in these patients (13,40). Moreover, specific miRNA clusters have been extensively described in GCTs, and their role as biomarkers of the disease is currently under investigation. However, the specific target genes whose transcription is regulated by these miRNAs are still unclear (41-43).

### Cisplatin resistant GCTs

Cisplatin-resistant or refractory GCTs are characterized by an increased DNA repair function to prevent chemotherapy-induced apoptosis. Most of the cisplatin-sensitive TCGT cell lines are unable to assemble Rad51 foci, which indicates a defect in initiating homologous recombination (HR) repair and explains the high sensitivity to cisplatin. On the other hand, platinum resistance is likely related to high *TP53* mutation rates and/or high tumor mutational burden (TMB) (33,40,44).

Recent studies have shown that mutations of *TP53*, *PIK3CA*, *AKT1*, *FGFR3*, *BRAF V600E*, *RAS*, and *FGFR3* are correlated to cisplatin resistance. *KRAS* and *NRAS* mutations have been described in 8/11 (72.7%) and 3/4 patients (75%) of cisplatin-resistant NSGCTs (but not in seminoma) (33,45,46). In a patient, who has both PMGCT and AML-M7 has shown to have *TP53* and *PTEN*

mutations in both tumor types which were reported to be associated with treatment resistance and poor prognosis (47).

*RAC1*, a member of the GTPase family, has analogous parts with *RAS* and plays a role in cell growth, migration (Sertoli cells), and therapy resistance in TGCCTs. Although *RAC1* mutation is rare, 5% was the highest rate declared so far in the general GCT tumor cohort (33,48).

### Genomic landscape of PMGCTs

The genomic landscape of PMGCTs is characterized by higher TMB and specific pathogenic alterations in known oncogenes or oncosuppressors involved in cancer development and progression compared to TGCTs. The most common mutations observed in PMGCTs are: *TP53* (46%), *c-KIT* (18%), *KRAS* (18%), *PTEN* (%11), *NRAS* (4%), and *PIK3CA* (4%). NS-PMGCT presents a higher TMB than NS-TGCTs. In a small retrospective study, 11.4% of the NS-PMGCTs harbored ≥10 mutations per Mb *vs.* 4.66% of the NS-TGCT (8,26,49).

In general, PMGCTs have a greater likelihood of having yolk sac differentiation and *TP53* alterations. Multivariate analysis considering the IGCCC prognostic risk factors demonstrated that *TP53/MDM2* alterations are independent negative prognostic factors for progressive disease after first-line cisplatin-based chemotherapy in NS-PMGCTs (8,50). In a retrospective study, *TP53* alterations were found in 72.2% of NS-PMGCT (13 of 18 patients), while none of the S-PMGCT patients harbored a *TP53* mutation. In the same study, *MYCN* amplification was described in 5 of 104 (4.8%) patients with cisplatin resistance and was almost mutually exclusive with *TP53/MDM2* mutations (51). *TP53* and *MDM2* are under the direct transcriptional regulation of *MYCN*. Small molecules (i.e., Nutlin) targeting the MDM2-TP53 interaction were demonstrated to sensitize testicular cells to cisplatin chemotherapy and induce apoptosis (52).

When compared to NS-TGCTs and seminomas (both gonadal and extragonadal), NS-PMGCTs have higher genomic alterations in *TP53* (P<0.0001), PIK3CA pathway (*PIK3CA*, *mTOR*, *PTEN*, *AKT1/2*) (P<0.0001), and cell-cycle regulation genes (*CCND1/2/3*, *CDK4/6*, *CDKN2A/B*, *RB1*) (P=0.0004). Other common tumor genomic alterations, including RAS-RAF (*KRAS*, *NRAS*, *HRAS*, *BRAF*), RTK (*ERBB2*, *PDGFRA*, *KIT*, *MET*, *FGFR 1/2/3*), and DDR (*BRCA1/2*, *ATM*, *CHEK2*, *MUTYH*), are not significantly different between NS-PMGCTS and S-PMGCTS and TGCTS (8).

The genomic profile described above could explain the worse prognosis observed in NS-PMGCTs versus TGCTs and S-PMGCTs. As the prognosis of NS-PMGCTs remains poor even in the modern era, new drugs and more effective first-line or salvage therapy regimens are needed. All the clinical trials using single-agent immune checkpoint inhibitors in cisplatin-resistant GCTs have failed to demonstrate a clinically meaningful activity (53). Of note, all these studies were conducted on unselected patients. Since NS-PMGCTS are among the GCTs with the highest TMB, even if single-agent immunotherapies did not show meaningful effects, future studies evaluating immunotherapies combined with platin-based chemotherapy and novel therapies such as demethylating agents could be studied in this rare subset. In this aspect, the comprehensive analysis of current data will help to provide a theoretical background for further research and explore druggable targets. It is also possible to explore biomarkers related to therapy response for immunotherapies.

## Association of PMGCTs with HMs and risk of malignant transformation

HMs are seen in approximately 2–3% of NS-PMGCT patients (54). The HMs can be simultaneously diagnosed with NS-PMGCTs (in 31% of the cases) or developed after the initial diagnosis of NS-PMGCTs (in 46% of the cases). The most common HM associated with NS-PMGCT is acute megakaryoblastic leukemia (AML-M7), followed by other types of AML, chronic myeloid leukemia, myelodysplasia, malignant mastocytosis, essential thrombocytopenia, and malignant histiocytosis. Interestingly, the accompanying HM cells are positive for i(12p) (shown in about 47% of PMGCT related HMs) and other GCTs specific mutations such as *TP53*, *KRAS*, and *PTEN*, suggesting that they derive from a common GCT progenitor.

Conversely, the lack of typical alterations for primary HMs such as *MLL* rearrangements or *FLT3* mutation further indicates a different etiopathogenesis of these malignancies (55). Moreover, in PMGCTs with associated HMs, *TP53* is the most frequently altered gene described in 90% of the patients and correlated to therapy resistance and poor prognosis (56,57). Additionally, *KRAS/NRAS* alterations are seen in 63% of PMGCT with concomitant HM, while *PTEN* mutation rates are similar between PMGCTs with or without HM (57,58).

Some studies have recently suggested that the HM

cells arise from resistant vasculogenic areas selected by chemotherapy. Although these theories unveil certain aspects of the HM associated with PMGCTs, further studies are needed to clarify the molecular associations of these tumors (59).

Besides HMs, somatic-type malignancies such as rhabdomyosarcoma, primitive neuroectodermal tumor (PNET), adenocarcinoma, and Wilms tumor can also be seen in association with PMGCTs and NS-TGCTs (60,61). Most of these tumors are believed to arise from pluripotent teratoma cells. Analysis of tumor samples showed chromosome 12p amplification, i(12p), and identical patterns of loss of heterozygosity in almost all of the somatic malignances associated to GCTs which suggest a common origin. Interestingly, tumor mutations specific of the transformed malignances have also been observed, such as t(11;12) characteristic of the PNET (61-63). As these tumors exhibit poor responses to cisplatin-based chemotherapy, surgery is the first and most effective treatment. The chemotherapy regimen should be selected on the base of the somatic transformation (64).

## Conclusions

In summary, NS-PMGCTs have a distinct genomic landscape characterized by a higher TMB and higher prevalence of *P53* mutations than TGCTs and S-PMGCT which could explain their lower sensitivity to cisplatin chemotherapy and poorer prognosis. Although some studies have unveiled recurrent molecular alterations in PMGCTs, few are suitable for targeted therapy. Due to the rarity of PMGCTs, the design of clinical trials in this patient population is extremely challenging. Data sharing and the creation of international collaborations would be helpful to have a better understanding of the molecular drivers of these tumors to improve patients selection and care.

## Acknowledgments

## Footnote

## References

1. Gaddam SJ, Chesnut GT. Testicle Cancer. [Updated 2021 Oct 9]. In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2021. Available online: https://www.ncbi.nlm.nih.gov/books/NBK563159/
2. Khan O, Protheroe A. Testis cancer. Postgrad Med J 2007;83:624-32.
3. Cheng L, Albers P, Berney DM, et al. Testicular cancer. Nat Rev Dis Primers 2018;4:29.
4. Bokemeyer C, Nichols CR, Droz JP, et al. Extragonadal germ cell tumors of the mediastinum and retroperitoneum: results from an international analysis. J Clin Oncol 2002;20:1864-73.
5. Stang A, Trabert B, Wentzensen N, et al. Gonadal and extragonadal germ cell tumours in the United States, 1973-2007. Int J Androl 2012;35:616-25.

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6. Napieralska A, Majewski W, Osewski W, et al. Primary mediastinal seminoma. J Thorac Dis 2018;10:4335-41.

7. Nichols CR. Mediastinal germ cell tumors. Semin Thorac Cardiovasc Surg 1992;4:45-50.

8. Necchi A, Bratslavsky G, Chung J, et al. Genomic Features for Therapeutic Insights of Chemotherapy-Resistant, Primary Mediastinal Nonseminomatous Germ Cell Tumors and Comparison with Gonadal Counterpart. Oncologist 2019;24:e142-5.

9. Voss MH, Feldman DR, Bosl GJ, et al. A review of second-line chemotherapy and prognostic models for disseminated germ cell tumors. Hematol Oncol Clin North Am 2011;25:557-76, viii -ix.

10. Pauniaho SL, Salonen J, Helminen M, et al. The incidences of malignant gonadal and extragonadal germ cell tumors in males and females: a population-based study covering over 40 years in Finland. Cancer Causes Control 2012;23:1921-7.

11. Oosterhuis JW, Looijenga LHJ. Human germ cell tumours from a developmental perspective. Nat Rev Cancer 2019;19:522-37.

12. De Felici M, Klinger FG, Campolo F, et al. To Be or Not to Be a Germ Cell: The Extragonadal Germ Cell Tumor Paradigm. Int J Mol Sci 2021;22:5982.

13. Shen H, Shih J, Hollern DP, et al. Integrated Molecular Characterization of Testicular Germ Cell Tumors. Cell Rep 2018;23:3392-406.

14. Urbini M, Schepisi G, Bleve S, et al. Primary Mediastinal and Testicular Germ Cell Tumors in Adolescents and Adults: A Comparison of Genomic Alterations and Clinical Implications. Cancers (Basel) 2021;13:5223.

15. Bokemeyer C, Droz JP, Horwich A, et al. Extragonadal seminoma: an international multicenter analysis of prognostic factors and long term treatment outcome. Cancer 2001;91:1394-401.

16. Rosti G, Secondino S, Necchi A, et al. Primary mediastinal germ cell tumors. Semin Oncol 2019;46:107-11.

17. Williams LA, Pankratz N, Lane J, et al. Klinefelter syndrome in males with germ cell tumors: A report from the Children's Oncology Group. Cancer 2018;124:3900-8.

18. Sowithayasakul P, Sinlapamongkolkul P, Treetipsatit J, et al. Hematologic Malignancies Associated With Mediastinal Germ Cell Tumors: 10 Years' Experience at Thailand's National Pediatric Tertiary Referral Center. J Pediatr Hematol Oncol 2018;40:450-5.

19. Mukherjee S, Ibrahimi S, John S, et al. Non-seminomatous mediastinal germ cell tumor and acute megakaryoblastic leukemia. Ann Hematol 2017;96:1435-9.

20. Baroni T, Arato I, Mancuso F, et al. On the Origin of Testicular Germ Cell Tumors: From Gonocytes to Testicular Cancer. Front Endocrinol (Lausanne) 2019;10:343.

21. Sheikine Y, Genega E, Melamed J, et al. Molecular genetics of testicular germ cell tumors. Am J Cancer Res 2012;2:153-67.

22. Nettersheim D, Schorle H. The plasticity of germ cell cancers and its dependence on the cellular microenvironment. J Cell Mol Med 2017;21:1463-7.

23. Litchfield K, Summersgill B, Yost S, et al. Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. Nat Commun 2015;6:5973.

24. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153-8.

25. Barrett MT, Lenkiewicz E, Malasi S, et al. Clonal analyses of refractory testicular germ cell tumors. PLoS One 2019;14:e0213815.

26. Nappi L, Annala M, Vandekerkhove G, et al. Molecular dissection of primary mediastinal germ cell tumors. J Clin Oncol 2017;35:417.

27. Atkin NB, Baker MC. Specific chromosome change, i(12p), in testicular tumors? Lancet 1982;2:1349.

28. Summersgill B, Goker H, Weber-Hall S, et al. Molecular cytogenetic analysis of adult testicular germ cell tumors and identification of regions of consensus copy number change. Br J Cancer 1998;77:305-13.

29. Martinez G, Kherraf ZE, Zouari R, et al. Whole-exome sequencing identifies mutations in FSIP2 as a recurrent cause of multiple morphological abnormalities of the sperm flagella. Hum Reprod 2018;33:1973-84.

30. Litchfield K, Levy M, Orlando G, et al. Identification of 19 new risk loci and potential regulatory mechanisms influencing susceptibility to testicular germ cell tumor. Nat Genet 2017;49:1133-40.

31. Willmore-Payne C, Holden JA, Chadwick BE, et al. Detection of c-kit exons 11- and 17-activating mutations in testicular seminomas by high-resolution melting amplicon analysis. Mod Pathol 2006;19:1164-9.

32. Kemmer K, Corless CL, Fletcher JA, et al. KIT mutations are common in testicular seminomas. Am J Pathol 2004;164:305-13.

33. Loveday C, Litchfield K, Proszek PZ, et al. Genomic landscape of platinum-resistant and sensitive testicular cancers. Nat Commun 2020;11:2189.

34. Cheng ML, Donoghue MTA, Audenet F, et al. Germ Cell Tumor Molecular Heterogeneity Revealed Through

Analysis of Primary and Metastasis Pairs. JCO Precis Oncol 2020;4:ePO.

35. Ferguson L, Agoulnik AI. Testicular cancer and cryptorchidism. Front Endocrinol (Lausanne) 2013;4:32.

36. Einhorn LH, Brames MJ, Heinrich MC, et al. Phase II study of imatinib mesylate in chemotherapy-refractory germ cell tumors expressing KIT. Am J Clin Oncol 2006;29:12-3.

37. Wermann H, Stoop H, Gillis AJ, et al. Global DNA methylation in fetal human germ cells and germ cell tumours: association with differentiation and cisplatin resistance. J Pathol 2010;221:433-42.

38. Buljubašić R, Buljubašić M, Bojanac AK, et al. Epigenetics and testicular germ cell tumors. Gene 2018;661:22-33.

39. Albany C, Fazal Z, Singh R, et al. A phase 1 study of combined guadecitabine and cisplatin in platinum refractory germ cell cancer. Cancer Med 2021;10:156-63.

40. Cavallo F, Graziani G, Antinozzi C, et al. Reduced proficiency in homologous recombination underlies the high sensitivity of embryonal carcinoma testicular germ cell tumors to Cisplatin and poly (adp-ribose) polymerase inhibition. PLoS One 2012;7:e51563.

41. Dieckmann KP, Radtke A, Spiekermann M, et al. Serum Levels of MicroRNA miR-371a-3p: A Sensitive and Specific New Biomarker for Germ Cell Tumours. Eur Urol 2017;71:213-20.

42. Mego M, van Agthoven T, Gronesova P, et al. Clinical utility of plasma miR-371a-3p in germ cell tumors. J Cell Mol Med 2019;23:1128-36.

43. Nappi L, Thi M, Lum A, et al. Developing a Highly Specific Biomarker for Germ Cell Malignancies: Plasma miR371 Expression Across the Germ Cell Malignancy Spectrum. J Clin Oncol 2019;37:3090-8.

44. Richardson C, Stark JM, Ommundsen M, et al. Rad51 overexpression promotes alternative double-strand break repair pathways and genome instability. Oncogene 2004;23:546-53.

45. Feldman DR, Iyer G, Van Alstine L, et al. Presence of somatic mutations within PIK3CA, AKT, RAS, and FGFR3 but not BRAF in cisplatin-resistant germ cell tumors. Clin Cancer Res 2014;20:3712-20.

46. Woldu SL, Amatruda JF, Bagrodia A. Testicular germ cell tumor genomics. Curr Opin Urol 2017;27:41-7.

47. Lu C, Riedell P, Miller CA, et al. A common founding clone with TP53 and PTEN mutations gives rise to a concurrent germ cell tumor and acute megakaryoblastic leukemia. Cold Spring Harb Mol Case Stud 2016;2:a000687.

48. Heinrich A, Potter SJ, Guo L, et al. Distinct Roles for Rac1 in Sertoli Cell Function during Testicular Development and Spermatogenesis. Cell Rep 2020;31:107513.

49. McIntyre A, Summersgill B, Spendlove HE, et al. Activating mutations and/or expression levels of tyrosine kinase receptors GRB7, RAS, and BRAF in testicular germ cell tumors. Neoplasia 2005;7:1047-52.

50. Timmerman DM, Eleveld TF, Gillis AJM, et al. The Role of TP53 in Cisplatin Resistance in Mediastinal and Testicular Germ Cell Tumors. Int J Mol Sci 2021;22:11774.

51. Bagrodia A, Lee BH, Lee W, et al. Genetic Determinants of Cisplatin Resistance in Patients With Advanced Germ Cell Tumors. J Clin Oncol 2016;34:4000-7.

52. Koster R, Timmer-Bosscha H, Bischoff R, et al. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis 2011;2:e148.

53. Adra N, Einhorn LH, Althouse SK, et al. Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: a Hoosier Cancer Research Network Study GU14-206. Ann Oncol 2018;29:209-14.

54. Nichols CR, Roth BJ, Heerema N, et al. Hematologic neoplasia associated with primary mediastinal germ-cell tumors. N Engl J Med 1990;322:1425-9.

55. Nichols CR. Malignant Hematologic Disorders Arising from Mediastinal Germ Cell Tumors. A Review of Clinical and Biologic Features. Leuk Lymphoma 1991;4:221-9.

56. Taylor J, Donoghue MT, Ho C, et al. Germ cell tumors and associated hematologic malignancies evolve from a common shared precursor. J Clin Invest 2020;130:6668-76.

57. Akizuki K, Sekine M, Kogure Y, et al. TP53 and PTEN mutations were shared in concurrent germ cell tumor and acute megakaryoblastic leukemia. BMC Cancer 2020;20:5.

58. Amra N, Zarate LV, Punia JN, et al. Mediastinal Germ Cell Tumor and Acute Megakaryoblastic Leukemia With Co-occurring KRAS Mutation and Complex Cytogenetics. Pediatr Dev Pathol 2020;23:461-6.

59. Levy DR, Agaram NP, Kao CS, et al. Vasculogenic Mesenchymal Tumor: A Clinicopathologic and Molecular Study of 55 Cases of a Distinctive Neoplasm Originating From Mediastinal Yolk Sac Tumor and an Occasional Precursor to Angiosarcoma. Am J Surg Pathol 2021;45:463-76.

60. Ehrlich Y, Beck SDW, Ulbright TM, et al. Outcome analysis of patients with transformed teratoma to primitive neuroectodermal tumor. Ann Oncol 2010;21:1846-50.

61. Domínguez Malagón H, Pérez Montiel D. Mediastinal germ cell tumors. Semin Diagn Pathol 2005;22:230-40.
62. Kernek KM, Brunelli M, Ulbright TM, et al. Fluorescence in situ hybridization analysis of chromosome 12p in paraffin-embedded tissue is useful for establishing germ cell origin of metastatic tumors. Mod Pathol 2004;17:1309-13.
63. Kum JB, Ulbright TM, Williamson SR, et al. Molecular genetic evidence supporting the origin of somatic-type malignancy and teratoma from the same progenitor cell. Am J Surg Pathol 2012;36:1849-56.
64. Donadio AC, Motzer RJ, Bajorin DF, et al. Chemotherapy for teratoma with malignant transformation. J Clin Oncol 2003;21:4285-91.