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REVIEW ARTICLE

New Anti-Cancer Strategies in Testicular Germ Cell Tumors

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Abstract: ***Background***: The most common solid malignancy of young men aged 20 to 34 years is testicular germ cell tumor. In addition, the incidence of these tumors has significantly increased throughout the last years. Testicular germ cell tumors are classified into seminoma and nonseminoma germ cell tumors, which take in yolk sac tumor, embryonal cell carcinoma, choriocarcinoma, and teratoma. There are noteworthy differences about therapy and prognosis of seminomas and nonseminoma germ cell tumors, even though both share characteristics of the primordial germ cells.

Objectives: The study is focused on different molecular mechanisms strongly involved in testicular germ cell line tumors underlying new strategies to treat this human neoplasia.

Methods: Bibliographic data from peer-reviewed research, patent and clinical trial literature, and around eighty papers and patents have been included in this review.

Results: Our study reveals that several biomarkers are usefully utilized to discriminate among different histotypes. Moreover, we found new patents regarding testicular germ cell tumor treatments such as the expression of claudin 6, monoclonal antibody (Brentuximab Vedotin), immune checkpoint blockade (ICB) with the FDA-approved drugs pembrolizumab and nivolumab or the oncolytic virus Pelareorep, the combination of selective inhibitors of Aurora kinase.

Conclusion: Finally, the pathogenesis of testicular germ cell tumor needs to be deeply understood so that it will improve data on stem cells, tumorigenesis and disease tumor management by more selective treatment.

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1. INTRODUCTION

Testicular Germ Cell Tumors (TGCTs) have the highest incidence among young men (between 20 and 34 years of age) solid tumors, and their incidence has improved over the last decades. About 90% of TGCTs are successfully treated with cisplastin-based chemotherapy. However, this kind of therapy raises the possibility of developing secondary cancers and cardiovascular disease.

TGCTs are classified into two principal groups: Germ Cell Neoplasias *In Situ* (GCNIS) that are Seminoma and Nonseminoma (NSE), and spermatocytic tumors that are not GCNIS. NSE tumors encompass embryonal carcinoma, choriocarcinoma, Yolk Sac Tumors (YSTs) and teratoma. TGCTs may develop from a non-invasive type of tumor called carcinoma *in situ* (CIS): Microscope analysis reveals abnormal cells even though they are still confined inside the membrane of the seminiferous tubules.

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The diagnosis of CIS is very hard due to the absence of side effects, however, the best way to detect CIS is through biopsy. In accord with its progression, three stages of the tumor can be determined: Stage I (the tumor is restricted into the testicle), stage II (the tumor has diffused to the abdominal lymph nodes) and stage III (the tumor has diffused to distant lymph nodes giving rise to metastases in organs such as liver and lungs). Moreover, the up-to-date classification of the urinary tract and male genital organs by World Health Organization (WHO) introduced adjournments about GCNIS that currently has been used as a new name for the precursor lesion (Fig. **1**) [1-6].

TGCT is a disease that derives from a wrong germ cell differentiation, and almost all TGCTs arise from defective foetal germ cells. In particular, recent studies underline the role of Primordial Germ Cells (PGCs) in TGCT pathophysiology [1-7]. Surgery and chemotherapy are the primary treatments for NSE tumors, and therapy success is strictly linked to the disease stage [8]. The cure rate is 99% in the first stage of NSE tumors, while in late disease stages it declines from 90% to 50% in poor prognostic patients [8]. Early lymph node metastases and/or distant metastases are

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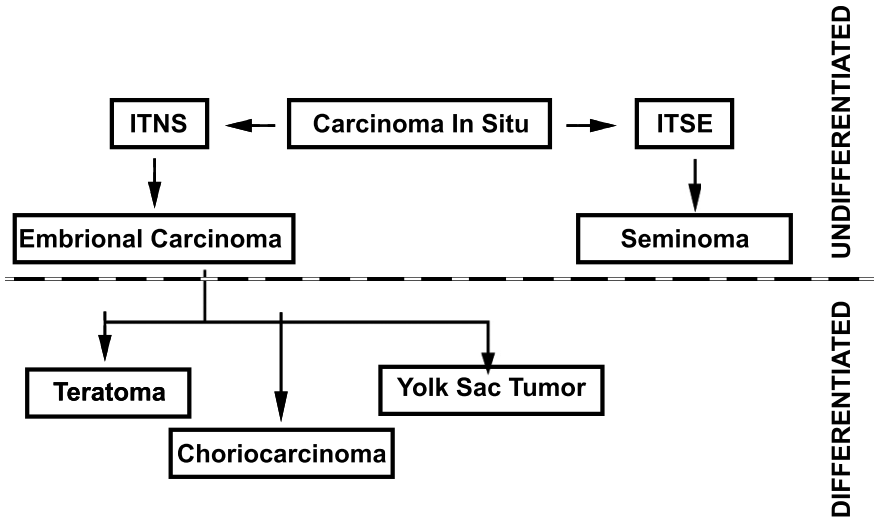

Fig. (1). Schematic representation of TGCT histotypes. ITNS = intratubular non seminoma; ITSE = intratubular seminoma.

frequently found in about 25% of patients with seminoma and up to 60% of those with nonseminoma due to the rapid progression and the fast growth of postpuberal TGCTs [8-10]. Although a lot of TGCTs therapeutic successes have been achieved in last decades, 10-20% of patients with metastatic disease do not reach a complete and durable remission after primary treatment, due to both incomplete response and/or a tumor relapse. This mini-review speculates about the new anticancer therapies in testicular germ cell tumors.

2. NOVEL MOLECULAR TARGETS IN TGCT SUBTYPES

Recently, numerous new biomarkers have been found to discriminate the TGCTs subtypes, standing for innovative molecular therapeutic targets. High-mobility group proteins A1 (HMGA1) and A2 (HMGA2) act for powerful diagnostic markers [11-14]. These two proteins are diversely expressed in TGCTs in comparison with the stage of tumor differentiation [11, 12]. For example, HMGA1 binds to other proteins, such as RNF4 [15, 16] and PATZ1, which are engaged in transcriptional control and have been demonstrated to be overexpressed and delocalized in human testicular seminomas [17]. Currently, we have shown that in human testicular seminomas Oestrogen Receptor β (ERβ) expression is strongly downregulated and this downregulation is associated with delocalization of both PATZ1 and HMGA1 transcriptional factors, on the contrary, in normal germ cells, PATZ1 binds to ERβ [18, 19].

The serine / threonine kinase NEK2 is a key regulator of the centrosome separation and bipolar spindle formation during mitosis and chromatin condensation during meiosis. It controls centrosome separation (essential for the formation of bipolar spindles and high-fidelity chromosome separation) through the phosphorylation of proteins such as CEP250, CROCC and NINL, causing their dislocation from the centrosomes. Additionally, NEK2 has the main function in chromatin condensation in the first meiotic division by HMGA2 phosphorylation [20]. Moreover, the enhancement and the nuclear localization of NEK2 protein have been found in both seminomas and in seminoma cell line (TCam-2) [21, 20]. Furthermore, recent studies underlined the new splicing factor kinase function of NEK2 [22, 23].

The Octamer-Binding Transcription Factor 3/4 (OCT3/4) transcription factor has been well described as a crucial regulator of pluripotency [24], representing a molecular marker for PGCs, GCNIS, embryonal carcinoma and seminoma [24]. OCT3/4 has also been found in normal adult stem cells and non-germ derived cancers cells [24]. Furthermore, other embryonic stem cell-specific proteins (i.e. NANOG and SOX2) are central for the maintenance of the so-called ‘‘stemness’’ of pluripotent cells in addition to OCT3/4, [25, 26]. Importantly, the OCT3/4 interaction with p53 (that is not mutated in TGCTs) may play a significant role in the chemotherapy response of these tumors [27].

The RNA-binding protein LIN28 is implicated in the maintenance of the pluripotency of embryonic stem cells, and its expression levels are reduced throughout the differentiation. In particular, LIN28 regulates the expression of OCT4 through directly binding to its mRNA transcript, in mouse embryonic stem cells. Indeed, LIN28 has a pivotal function for reprogramming somatic cells into pluripotent stem cells. Moreover, LIN28 represents a valid diagnostic marker for testicular GCNIS, classical seminomas, embryonal carcinomas, and YSTs [28]. In particular, LIN28 is the main YST marker due to the absence of OCT4 [28].

NANOG protein has been found in gonocytes localized in developing testis [26]. Furthermore, it is highly and unambiguously expressed only in GCNIS, embryonal carcinoma, and seminomas, but not in teratoma, and YST tumour underlining a developmental and molecular connection between TGCTs and the embryonic cells from which they have origin [29].

The SOX protein family consists of transcription factors working during the development of specific cells from the first embryonal stages to more differentiated phenotypes. SOX protein family collaborates with Pituitary Octamer Unc-86 (POU) transcription factor proteins; among the member of SOX and POU families, the most important interaction is between OCT3/4 and SOX2. SOX2, which is absent in normal human germ cells, is expressed in mouse

embryos [30] and in embryonal carcinoma, but not in YSTs and seminomas [30]. In SOX protein family, SOX17, which maps to the chromosomal region 8p23, which is gained in seminoma, represents a good candidate for the interaction with OCT3/4 in human intratubular germ cell neoplasia (IGCNU) and seminoma. Consistently, SOX17 may be considered a valuable marker to distinguish CIS and seminoma from embryonal carcinoma. Moreover, SOX17 is a helpful diagnostic marker to discriminate between embryonic and adult hematopoietic stem cells [31]. Recent studies aim to better understand the processes that control the SOX2 versus SOX17 differential expression and the role of SOX proteins in the diverse morphologies of the TGCT subtypes involved.

Until recently, the Oestrogen Receptors α (ERα) and Oestrogen Receptors β (ERβ) [32, 33] have been considered the major physiologic oestrogen mediators. Indeed, the G Protein-Coupled Oestrogen Receptor (GPR30) is acquiring an increasingly vital role in oestrogen-mediated signalling in a wide variety of cell types. The critical role of GPR30 in the preservation and in the development and homeostasis of the normal testis is well recognized [34, 35]. Recent studies show that GPR30 is overexpressed in human spermatogonia, spermatocytes [35], in TCam-2 cell line and seminomas. Moreover, it has been verified that the ERβ downregulation correlates with GPR30 overexpression both in human CIS and seminomas; furthermore, it has been demonstrated that 17β-oestradiol produces the ERK1/2 activation through GPR30 [35]. Many studies are committed to develop a novel therapeutic strategy for the treatment of TGCTs blocking neoplastic germ cells through the design of selective GPR30 inhibitors [36, 37].

It has been extensively illustrated that DNA Damage Response (DDR) pathway acts as an anti-cancer apparatus in early human carcinogenesis. Remarkably, TGCTs have been shown to be defective with respect to DDR pathway activation. For instance, the pro-apoptotic protein CCDC6, substrate of the Ataxia Telangectasia Mutated (ATM), acts as DNA damage checkpoint following genotoxic stress and is frequently rearranged in cancer upon fusion with several molecular partners. As reported in different papers, in TGCTs and human seminoma cell line the loss of CCDC6 expression is very common [38].

Not long ago, the DNA damage levels in Peripheral Blood Lymphocytes (PBLs) of TGCT patients has been proposed as a prognostic marker for identifying patients with poor outcome [39].

The modification of Poly (ADP-Ribose) Polymerase-1 (PARP-1) has also been found in TGCTs; in addition, it has been demonstrated that the absence of PARP-1 stimulates trophoblast differentiation from mouse embryonic stem cells in the formation of teratocarcinoma-like tumor [40, 41]. Over 80% of metastatic TGCTs is successfully treated with Cisplatin-based chemotherapy. On the contrary, almost all other solid cancers are incurable as soon as they spread outside the primary site. Cell lines originated from TGCTs are hypersensitive to cisplatin likely due to their decreased repair ability, even though the key damage to DNA has not been identified. Today, the exceptional sensitivity of Testis Tumour Cells (TTC) and their curability of TGCT has been related to the limited Interstrand Crosslinks (ICL) repair due to ERCC1-XPF low expression [42, 43].

The enzyme XRCC1 is an essential element of the Base Excision Repair (BER) pathway and Double Strand Break Repair (DSBR) pathway. Interestingly, it has been demonstrated that the susceptibility and chromosomal aberration of TGCTs associates with XRCC1 gene polymorphisms [44]. Moreover, it has been shown that two patients, which were refractory to the treatment, harboured XRCC2 mutations, a gene clearly linked to cisplatin resistance [45].

Adult testicular germ cells normally express Cancer Testis (CT) antigens. In cancer patients, the expression of chromosome X-encoded CT (CT-X) antigens was originally found searching for immunogenic tumor antigens able to causing spontaneous immune reactions. Analysis of B Melanoma Antigen (BAGE), Melanoma Associated Antigen (MAGE) and G Antigen 1 (GAGE), antigens which are direct cell-mediated immune responses in melanoma patients, demonstrated that their expression was not found in normal adult tissue but limited to testis. Furthermore, it has been described that in the human foetal gonad stage 15 weeks a very few positive MAGE-A4 cells appear and strong staining was detected in gonocytes of the foetal gonad stage 28 weeks [43]. Then, SSX, NY-ESO and CT7 genes were identified by serological cloning of antigens that caused antibody responses in cancer patients. All of them have distinctive characteristics of testis confined expression and atypical activation in various human cancers. This exclusive characteristic permits to select this set of antigens as CT antigens, identifying them as suitable targets for immunotherapy, mostly for therapeutic cancer vaccines [46].

Not long ago, it has been shown that CT-X antigens are absent in foetal precursor cells for germ cell tumors. Indeed, their expression seems to mirror germ cell differentiation of neoplastic cells (in seminomas) or atypical gene activation as tumor antigens (in nonseminomatous cancers) [46]. Finally, TGCT susceptibility has been connected with KITLG (c-KIT ligand) through genome-wide studies. In fact, KITLG controls the development of primordial germ cells, from which TGCT is thought to arise and spermatogenesis advances [47].

3. AURORA B KINASE INHIBITORS

Cancer is often caused by the accumulation of genetic anomalies in several regulators of cell cycle [48]. More in deep, in proliferating tissues, Aurora B expression and activity are strictly cell-cycle controlled, reaching the expression peak at the G2-M transition, whereas its maximum kinase activity is achieved during mitosis. Aurora B phosphorylates serine 10 of histone H3, a pivotal modification involved in chromosome condensation, alignment and segregation. Aurora B is also essential for spindle checkpoint function and cytokinesis [48]. Several studies have found that aneuploidy of TGCTs is strongly linked to centrosome amplification [48]. In TGCT diagnosis, Aurora B kinase is another helpful molecular marker having the ability to distinguish among the various tumor histotypes: it is well found in IGCNU, seminomas and embryonal carcinomas, but not in teratomas and YST tumor [49-53]. Importantly, the inhibition of Aurora B kinase activity considerably reduces cell growth

rate in testis-derived GC1 and TCam-2 cell lines [54]. Indeed, Aurora B inhibitors such as AZD1152, ZM447439, Hesperadin 8 and VX-680 have been recently tested [54, 55]. In particular, AZD1152 is more specific for the inhibition of Aurora kinase B than for Aurora kinase A, significantly blocking tumor growth in a wide panel of solid human cancer xenograft models. Importantly, AZD1152 and other Aurora B inhibitors (ZM2, ZM3, GSK1070916) show reversible neutropenia as a main side effect and they are in early clinical evaluation [54]. These inhibitors block the phosphorylation of histone H3 on serine 10 [55], and then blocking cell division. Although TGCTs are highly responsive to chemotherapeutic treatment, several studies reported acute toxicity and chronic collateral effects, such as sterility. Hence, the presence of innovative drugs represents a breakout from the side effects of chemotherapy.

4. NON-CODING RNAS IN TGCTS

MicroRNAs (miRNAs) are short non-coding RNAs and take part in the regulation of gene expression [56]. Thus, they have a pivotal role in cellular maintenance, development and differentiation. Perturbation of miRNAs plays an important part in the instauration and progression of many cancer types, including TGCTs [57]. Although different miRNA signatures are associated with histological subtypes of TGCT, very few miRNAs have been found to have a key role in TGCTs. Indeed, Dicer knockout mouse shows a premature reduction of germ cell numbers and deregulated differentiation of male germ cells [58]. Then, Voorhoeve *et al*. showed that miR-372 and miR-373 may overcome p53-mediated arrest of cell cycle [59]. Conversely, miR-372 and miR-373 were absent in TGCT-derived cell lines with mutated p53 or expressing low levels of p53, suggesting that these miRNAs may allow the growth of TGCT escaping the p53 checkpoint of cell-cycle. In this context, several data suggest that miR-372 and miR-373 may act as oncogenes in TGCT through the inhibition of LATS2, a tumor suppressor gene [59].

Moreover, a recent paper by Langroudi *et al.* has evidenced the involvement of miR-371-373 cluster in human Embryonic Stem Cells (ESCs) fate. Deeper, this miRNA cluster is prominently expressed in ESCs, whereas its expression levels are rapidly reduced after cell differentiation. This cluster likely acts by limiting the huge cell cycle propagation, protecting cells from replicative stress, and by activating CDK inhibitors and several retinoblastoma transcriptional repressors, causing cell cycle block [60]. These data underline the tumor suppressor role of miR-371-373 cluster. Recently, it is well reported that miR-514a-3p may act as an apoptotic activator by repressing PEG3 mRNA. In particular, PEG3 expression levels are increased in TGCTs, in which the expression of miR-514a-3p was lost, supporting a model of PEG3-mediated activation of NF-κB through the recruitment of TRAF2 and, consequently, the initiation the NF-κB pathway for protecting cells from apoptosis (Table **1**) [59-61].

Another interesting report underlining the importance of the connection between miRNAs and TGCTs suggests that the Dead end gene (DND1) is able to control germ-cell viability and to block germ cell tumors formation. Indeed, Kedde *et al.* described that DND1 may overcome miRNA-mediated degradation of transcripts by binding to mRNAs and avoiding the miRNA interaction with their target sites [62]. Furthermore, Linger *et al.* (2008) reported DND1 mutations in 263 human TGCTs [63], indicating the pivotal role of the fine miRNA pathway regulation in germ cell development.

Moreover, the novel identification of circulating miRNAs in body fluids like serum, may represent a valid non-invasive manner to diagnosis and follow the disease status. In this regard, it has been reported that miR-371 and miR-372 are specifically increased in the serum of germ cell tumors patients. Moreover, many other miRNAs have been proposed to be able to discriminate between the different tumor histotypes, confirming the function of the embryonic miR-371 and miR-372 in identifying malignant TGCT [64].

Pseudogenes have long been considered as non-functional genomic sequences. However, recent evidence suggests that many of them might have some form of biological activity, and the possibility of functionality by a microRNA-mediated pathway [65]. Recently, two HMGA1 processed pseudogenes (HMGA1P6 and HMGA1P7) were isolated. In particular, these pseudogenes, competing with HMGA1 for microRNA binding, lead to the upregulation of HMGA1 cellular levels, exerting an oncogenic role [66-71]. In this context, although further experiments are needed, preliminary data show that HMGA1 pseudogenes are differentially overexpressed in TGCT histotypes in comparison with normal testis (seminomas, embryonal carcinomas, mixed form, teratomas and YSTs), suggesting a role of HMGA1 pseudogenes in TGCT carcinogenesis.

Table 1. Main miRNAs Involved in TGCT Tumorigenesis.

microRNA	Function	Mechanism	Reference Number
Mir-372, mir-373	Oncogene	• Overcome p53-mediated arrest of cell cycle • Inhibition of LATS2	[59]
Mir-371-373 cluster	Tumor suppressor	• Limit cell cycle propagation • Activate CDK inhibitors • Induce retinoblastoma repressors	[60]
Mir-514a-3p	Tumor suppressor	• Act as apoptotic activator by repressing PEG3	[61]

Table 2. New Patented Treatment in TGCT.

Patent ID	Patent Title	Short Description	Reference Number
WO2012003956	Cancer therapy using CLDN6 target-directed antibodies *in vivo*	Antibodies to CLDN6 on the surface of tumor cells are able to inhibit growth of the tumor and to prolong survival of tumor patients	[74]
US8906376	Drug conjugates and their use for treating cancer, an autoimmune disease or an infectious disease.	Monoclonal antibodies used in patients whose cancer returned after high-dose chemotherapy	[75]
US13408478	Reovirus clearance of RAS-mediated neoplastic cells from mixed cellular compositions	Reovirus can be used to selectively remove RAS-mediated neoplastic cells	[76]
US20160193224	Methods of treating cancer using aurora kinase inhibitors	Treatment of various cell proliferative disorders	[77]

5. NOVEL TGCT PATENTED TREATMENTS

Although TGCTs have a good response to surgery and chemotherapy, some patients do not present a full remission. In last years several novel approaches to TGCT tumor have been found both to treat resistant patients and to prevent some permanent side effects (i.e. sterility). One novel patented invention to treat TGCT disease is based on the expression of Claudin 6 (CLDN6), a transmembrane protein that has been found deregulated in several human carcinomas [72, 73]. This new patented treatment proves that using antibodies against CLDN6 that is expressed on tumor cells surface blocks tumor growth, extending the lifespan of TGCT patients [74].

Moreover, another monoclonal antibody (Brentuximab Vedotin) is under evaluation (multicenter Phase II Study) in TGCT patients whose cancer returned after high-dose chemotherapy [75] (Table **2**) [74-77]. More in deep, Brentuximab vedotin is routinely used in lymphoma treatment as an anticancer drug. Its mechanism consists in two parts: One (the monoclonal antibody) that is directed against a cancer cell surface protein (CD30), and another (monomethyl auristatin E an anticancer drug) that eliminates cancer [75]. Other new methods are now starting to be used in TGCT clinical treatments such as immune checkpoint blockade (ICB) with the FDA-approved drugs pembrolizumab and nivolumab or the oncolytic virus Pelareorep, patented by Oncolytics Biotech Inc [76]. Moreover, additive and synergistic anti TGCT activities have been demonstrated for the combination of selective inhibitors of Aurora Akinase with taxanes [77] (Table **2**).

CONCLUSION

Genetic and environmental factors play a pivotal function in the cancerogenesis of human TGTCs likely acting as the regulator of the normal differentiation process in PGCs. However, during the last decades, the increasing knowledge of the different molecular mechanisms that lead to the instauration of TGCTs has yielded to several molecules that are currently used in therapy. Although, the majority of TGCTs are successfully treated with cisplastin-based chemotherapy, new therapies based on novel patented drugs will raise the possibility of developing secondary cancers and cardiovascular disease.

CURRENT AND FUTURE DEVELOPMENT

The development of human TGCTs is subjected to genetic and environmental factors that have a crucial role in deregulating the normal differentiation process in PGCs. Recently, the increasing number of tumor biomarkers has permitted the histological discrimination among the various subgroups. A better comprehension of the molecular pathways through which the TGCTs develop will point out new tools to definitely target cancer cells and will help to defeat intrinsic and acquired chemotherapy resistance. Aurora-B serine-threonine kinases, HMGAs and GPR30 inhibitors are promising molecules able to selectively target cancer cells introducing a new scenario for TGCTs treatment in the next future.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Chieffi P. Molecular targets for the treatment of testicular germ cell tumors. Mini Rev Med Chem 2007; 7: 755-9.

[2] Chieffi P, Franco R, Portella G. Molecular and cell biology of testicular germ cell tumors. Int Rev Cell Mol Biol 2009; 278: 277-308.

[3] Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs-part A: Renal, penile, and testicular tumours. Eur Urol 2016; 70: 93-105.

[4] Chieffi P, Chieffi S, Franco R, Sinisi A. Recent advances in the biology of germ cell tumors: Implications for the diagnosis and treatment. J Endocrinol Invest 2012; 35: 1015-20.

[5] Chieffi P, Chieffi S. Molecular biomarkers as potential targets for therapeutic strategies in human testicular germ cell tumours: An overview. J Cell Physiol 2013; 22: 1641-6.

[6] Chieffi P, Chieffi S. An up-date on newly discovered immunohistochemical biomarkers for the diagnosis of human testicular germ cell tumors. Histol Histopathol 2014; 29: 999-1006.

[7] Chieffi P. An overview on predictivebiomarkers of testiculargermcelltumors. J Cell Physiol 2017; 232: 276-80.

[8] Chieffi P. Potential new anticancermolecular targets for the treatment of human testicularseminomas. Mini Rev Med Chem 2011; 11: 1075-81.

[9] Shelley MD, Burgon K, Mason MD. Treatment of testicular germcell cancer: A cochrane evidence-based systematic review. Cancer Treat Rev 2002; 28: 237-53.

[10] Perrotti M, Ankem M, Bancilla A, deCarvalho V, Amenta, P, Weiss R. Prospective metastatic risk assignment in clinical stage I nonseminomatous germ cell testis cancer: a single institution pilot study. Urol Oncol 2004; 22: 174-7.

[11] Chieffi P, Battista S, Barchi M, Di Agostino S, Pierantoni G, Fedele M, *et al.* HMGA1 and HMGA2 proteinexpression in mouse spermatogenesis. Oncogene 2002; 21: 3644-50.

[12] Franco R, Esposito F, Fedele M, Liguori G, Pierantoni G, Botti G, *et al.* Detection of high mobility group proteins A1 and A2 represents a valid diagnostic marker in post-puberal testicular germ cell tumours. J Pathol 2008; 214: 58-64.

[13] Esposito F, Tornincasa M, Chieffi P, De Martino I, Pierantoni G, Fusco A. High mobility group A1 (HMGA1) proteins regulate p53-mediated transcription of Bcl-2 gene. Cancer Res 2010; 70: 5379-88.

[14] Federico A, Forzati F, Esposito F, Arra C, Palma G, Barbieri A, *et al.* HMGA1/HMGA2 double knock-out mice display a "super-pygmy" phenotype. Biol Open 2014; 3(5): 372-8.

[15] Pero R, Lembo F, Di Vizio D, Boccia A, Chieffi P, Fedele M, *et al.* RNF4 is a growthinhibitorexpressed in germcells and lost in human testicular tumours. Am J Pathol 2001; 159: 1225-30.

[16] Pero R, Lembo F, Chieffi P, Del Pozzo G, Fedele M, Fusco A, *et al.* Translationalregulation of a novel testis-specific RNF4 transcript. Mol Reprod Dev 2003; 66: 1-7.

[17] Fedele M, Franco R, Salvatore G, Paronetto MP, Barbagallo F, Pero R, *et al.* PATZ1 gene has a critical role in the spermatogenesis and testiculartumours. J Pathol 2008; 215: 39-47.

[18] Esposito F, Boscia F, Franco R, Tornincasa M, Fusco A, Kitazawa S, *et al.* Down-regulation of estrogen receptor-β associates with transcriptional coregulator PATZ1 delocalization in human testicular seminomas. J Pathol 2011; 224: 110-20.

[19] Esposito F, Boscia F, Gigantino V, Tornincasa M, Fusco A, Franco R, *et al.* The high mobility group A1-oestrogen receptor β nuclear interaction is impaired in human testicular seminomas. J Cell Physiol 2012; 227: 3749-55.

[20] Di Agostino S, Fedele M, Chieffi P, Fusco A, Rossi P, Geremia R, *et al.* Phosphorylation of high mobility group protein A2 by Nek kinase during the first meiotic division in mouse spermatocytes. Mol Biol Cell 2004; 15: 1224-32.

[21] Barbagallo F, Paronetto MP, Franco R, Chieffi P, Dolci S, Fry AM, *et al.* Increased expression and nuclear localization of the centrosomal kinase NEK2 in human testicular seminomas. J Pathol 2009; 217: 431-41.

[22] Naro C, Barbagallo F, Chieffi P, Bourgeois CF, Paronetto MP, Sette C. The centrosomal kinase NEK2 is a novel splicing factor kinase involved in cell survival. Nucleic Acids Res 2014; 42: 3218-27.

[23] Chieffi P, Chieffi S. An up-date on the molecular biomarkers as potential therapeutic targets in human testicular germ cell tumours. Open Androl J 2013; 5: 6-9.

[24] Atlasi Y, Mowla SJ, Ziaee SA, Gokhale PJ, Andrews PW. OCT4 spliced variants are differentially expressed in human pluripotent and non-pluripotent cells. Stem Cells 2008; 26: 3068-74.

[25] Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell BR. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev 2003; 17: 126-40.

[26] Yamaguchi S, Kimura H, Tada M, Nakatsuji N, Tada T. Nanog expression in mouse germ cell development. Gene Expr Patterns 2005; 5: 639-46.

[27] Voutsadakis IA. The chemosensitivity of testicular germ cell tumors. Cell Oncol 2014; 37: 79-94.

[28] Cao D, Allan RW, Cheng L, Peng Y, Guo CC, Dahiya N, *et al.* RNA-binding protein LIN28 is a marker for testicular germ cell tumors. Hum Pathol 2011; 42: 710-18.

[29] Hart AH, Hartley L, Parker K, Ibrahim M, Looijenga LH, Pauchnik M, *et al.* The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. Cancer 2005; 104: 2092-98.

[30] de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, Boer M, *et al.* Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. J Pathol 2008; 215: 21-30.

[31] Kim I, Saunders TL, Morrison SJ. Sox17 dependence distinguishes the transcriptional regulation of fetal from adult hematopoietic stem cells. Cell 2007; 130: 470-83.

[32] Vicini E, Loiarro M, Di Agostino S, Corallini S, Capolunghi F, Carsetti R, *et al.* 17-β-Estradiol elicits genomic and non-genomic responses in mouse male germ cells. J Cell Physiol 2006; 206: 238-45.

[33] Staibano S, Franco R, Mezza E, Chieffi P, Sinisi A, Pasquali D, *et al.* Loss of oestrogen receptor beta, high PCNA and P53 expression and aneuploidy as markers of worse prognosis in ovarian granulosa cell tumours. Histopathology 2003; 43: 254-62.

[34] Chieffi P, Colucci D'Amato GL, Staibano S, Franco R, Tramontano D. Estradiol-induced mitogen-activated protein kinase (extracellular signal-regulated kinase 1 and 2) activity in the frog (*Rana esculenta*) testis. J Endocrinol 2000; 167: 77-84.

[35] Franco R, Boscia F, Gigantino V, Marra L, Esposito F, Ferrara D, *et al.* GPR30 is over-expressed in post puberal testicular germ cell tumors. Cancer Biol Ther 2011; 11: 609-13.

[36] Boscia F, Passaro C, Gigantino V, Perdonà S, Franco R, Portella G, *et al.* High levels of GPR30 protein in human testicular carcinoma *in situ* and seminomas correlate with low levels of estrogen receptor-beta and indicate a switch in estrogen responsiveness. J Cell Physiol 2015; 230: 1290-97.

[37] Chieffi P, Franco R, Fulgione D, Staibano S. PCNA in the testis of the frog, *Rana esculenta*: A molecular marker of the mitotic testicular epithelium proliferation. Gen Comp Endocrinol 2000; 119: 11-16.

[38] Staibano S, Ilardi G, Leone V, Luise C, Merolla F, Esposito F, *et al.* Critical role of CCDC6 in the neoplastic growth of testicular germ cell tumors. BMC Cancer 2013; 13: 433-9.

[39] Sestakova Z, Kalavska K, Hurbanova L, Jurkovicova D, Gursky J, Chovanec M, *et al.* The prognostic value of DNA damage level in peripheral blood lymphocytes of chemotherapy-naïve patients with germ cell cancer. Oncotarget 2016; 7: 75996-6005.

[40] Shiokawa M, Masutani M, Fujihara H, Ueki K, Nishikawa R, Sugimura T, *et al.* Genetic alteration of Poly (ADP-ribose) polymerase-1 in human germ cell tumors. Jpn J Clin Oncol 2005; 35: 97-102.

[41] Mego M, Cierna Z, Svetlovska D, Macak D, Machalekova K, Miskovska V, *et al.* PARP expression in germ cell tumours. J Clin Pathol 2013; 66: 607-12.

[42] Usanova S, Piée-Staffa A, Sied U, Thomale J, Schneider A, Kaina B, *et al.* Cisplatin sensitivity of testis tumour cells is due to deficiency in inter strand-crosslink repair and low ERCC1-XPF expression. Mol Cancer 2010; 9: 248.

[43] Pauls K, Schorle H, Jeske W, Brehm R, Steger K, Wernert N, *et al.* Spatial expression of germ cell markers during maturation of human fetal male gonads: An immune histo chemical study. Hum Reprod 2006; 21: 397-404.

[44] Tsuchiya N, Mishina M, Narita J, Kumazawa T, Inoue T, Horikawa Y, *et al.* Association of XRCC1 gene polymorphisms with the sus-

ceptibility and chromosomal aberration of testicular germ cell tumors. Int J Oncol 2006; 28: 1217-23.

[45] Litchfield K, Summersgill B, Yost S, Broderick P, Nsengimana J, Eeles R, *et al.* Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. Nat Commun 2015; 6: 5973-81.

[46] Chen YT, Chiu R, Lee P, Beneck D, Jin B, Old LJ. Chromosome X-encoded cancer/testis antigens show distinctive expression patterns in developing gonads and in testicular seminoma. Hum Reprod 2011; 26: 3232-43.

[47] Ferlin A, Pengo M, Pizzol D, Carraro U, Frigo AC, Foresta C. Variants in KITLG predispose to testicular germ cell cancer independently from spermatogenic function. Endocr Relat Cancer 2012; 19: 101-8.

[48] Mayer F, Stoop H, Sen S, Bokemeyer C, Oosterhuis JW, Looijenga LHJ. Aneuploidy of human testicular germ cell tumors is associated with amplification of centrosomes. Oncogene 2003; 22: 3859-66.

[49] Chieffi P, Troncone G, Caleo A, Libertini S, Linardopoulos S, Tramontano D, *et al.* Aurora B expression in normal testis and seminomas. J Endocrinol 2004; 181: 263-70.

[50] Esposito F, Libertini S, Franco R, Abagnale A, Marra L, Portella G, *et al.* Aurora B expression in post-puberal testicular germ cell tumours. J Cell Physiol 2009; 221: 435-39.

[51] Portella G, Passaro C, Chieffi P. Aurora B: A new prognostic marker and therapeutic target in cancer. Curr Med Chem 2011; 18: 482-96.

[52] Marra L, Cantile M, Scognamiglio G, Marra L, Perdonà S, La Manti E, *et al.* Deregulation of HOXB13 expression in urinary bladder cancer progression. Curr Med Chem 2013; 20: 833-39.

[53] Franco R, Zappavigna S, Gigantino V, Luce A, Cantile M, Cerrone M, *et al.* Urotensin II receptor determines prognosis of bladder cancer regulating cell motility/ invasion. J Exp Clin Cancer Res 2014; 33: 48-57.

[54] Keen N, Taylor S. Aurora-kinase inhibitors as anticancer agents. Nat Rev Cancer 2004; 4: 927-36.

[55] Gavriilidis P, Giakoustidis A, Giakoustidis D. Aurora kinases and potential medical applications of Aurora kinase inhibitors: A review. J Clin Med Res 2015; 7: 742-51.

[56] Forzati F, De Martino M, Esposito F, Sepe R, Pellecchia S, Malapelle U, *et al.* miR-155 is positively regulated by CBX7 in mouse embryonic fibroblasts and colon carcinomas, and targets the KRAS oncogene. BMC Cancer 2017; 17(1): 170-8.

[57] Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, *et al.* Dicer is essential for mouse development. Nat Genet 2003; 35: 215-17.

[58] Maatouk DM, Loveland KL, McManus MT, Moore K, Harfe BD. Dicer1 is required for differentiation of the mouse male germline. Biol Reprod 2008; 79: 696-703.

[59] Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, *et al.* A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 2006; 124: 1169-81.

[60] Langroudi L, Jamshidi-Adegani F, Shafiee A, Rad SM, Keramati F, Azadmanesh K, *et al.* MiR-371-373 cluster acts as a tumor- suppressor-miR and promotes cell cycle arrest in unrestricted somatic stem cells. Tumour Biol 2015; 36: 7765-74.

[61] Özata DM, Li X, Lee L, Liu J, Warsito D, Hajeri P, *et al.* Loss of miR-514a-3p regulation of PEG3 activates the NF-kappa B pathway in human testicular germ cell tumors. Cell Death Dis 2017; 8: e2759.

[62] Kedde M, Strasser MJ, Boldajipour B, Oude Vrielink JA, Slanchev K, le Sage C, *et al.* RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. Cell 2007; 131: 1273-86.

[63] Linger R, Dudakia D, Huddart R, Tucker K, Friedlander M, Phillips KA, *et al.* Analysis of the DND1 gene in men with sporadic and familial testicular germ cell tumors. Genes Chrom Cancer 2008; 47: 247-52.

[64] Rijlaarsdam MA, van Agthoven T, Gillis AJM, Patel S, Hayashibara K, Lee KY, *et al.* Identification of known and novel germ cell cancer-specific (embryonic) miRs in serum by high-throughput profiling. Andrology 2015; 3: 85-91.

[65] Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 2010; 465(7301): 1033-8.

[66] Esposito F, De Martino M, Petti MG, Forzati F, Tornincasa M, Federico A, *et al.* HMGA1 pseudogenes as candidate proto-oncogenic competitive endogenous RNAs. Oncotarget 2014; 5(18): 8341-54.

[67] Esposito F, De Martino M, Forzati F, Fusco A. HMGA1-pseudogene overexpression contributes to cancer progression. Cell Cycle 2014; 13(23): 3636-9.

[68] Esposito F, De Martino M, D'Angelo D, Mussnich P, Raverot G, Jaffrain-Rea ML, *et al.* HMGA1-pseudogene expression is induced in human pituitary tumors. Cell Cycle 2015; 14(9): 1471-5.

[69] De Martino M, Forzati F, Arra C, Fusco A, Esposito F. HMGA1-pseudogenes and cancer. Oncotarget 2016; 7(19): 28724-35.

[70] De Martino M, Forzati F, Marfella M, Pellecchia S, Arra C, Terracciano L, *et al.* HMGA1P7-pseudogene regulates H19 and Igf2 expression by a competitive endogenous RNA mechanism. Sci Rep 2016; 6: 37622-9.

[71] De Martino M, Palma G, Azzariti A, Arra C, Fusco A, Esposito F. The HMGA1 pseudogene 7 induces miR-483 and miR-675 upregulation by activating EGR1 through a ceRNA mechanism. Genes (Basel) 2017; 8(11): 52-9.

[72] Lal-Nag M, Battis M, Santin AD, Morin PJ. Claudin-6: A novel receptor for CPE-mediated cytotoxicity in ovarian cancer. Oncogenesis 2012; 1: e33.

[73] Micke P, Mattsson JS, Edlund K, Lohr M, Jirström K, Berglund A, *et al.* Aberrantly activated claudin 6 and 18.2 as potential therapy targets in non-small-cell lung cancer. Int J Cancer 2014; 135(9): 2206-14.

[74] Sahin, U., Tureci, O., Koslowski, M., Walter, K., Kreuzberg, M., Luxen, S. Cancer therapy using CLDN6 target-directed antibodies *in vivo*. WO2012003956 (**2012**).

[75] Senter, P.D., Doronina, S., Toki, B.E. Drug conjugates and their use for treating cancer, an autoimmune disease or an infectious disease. US8906376 (**2010**).

[76] Morris, D., Thompson, B.G., Matthew, C., Coffey. Reovirus clearance of RAS-mediated neoplastic cells from mixed cellular compositions. US13408478 (**2012**).

[77] Ecsedy, JA., Shyu, WC., Chakravarty, A., Kleinfield, RW., Le, KN., Venkatakrishnan, K. Methods of treating cancer using aurora kinase inhibitors. US20160193224 (**2016**).