The incidence of testicular germ cell tumour (TGCT) is increasing and is a leading cause of death in young men. The introduction of cisplatin therapy 40 years ago resulted in improved survival for metastatic TGCT. Stratification of metastatic disease into good-risk, intermediate-risk, and poor-risk groups via the International Germ Cell Consensus Classification has, for the past 20 years, facilitated standardization of treatment and direct comparison of clinical trial results. However, escalation of therapy within the intermediate-risk and/or poor-risk groups has not generally resulted in clear survival advantages. Furthermore, attempts to identify additional clinical and/or tumour markers to predict platinum resistance have largely failed.

The end of 2016 saw publication of long-awaited landmark first insights into platinum resistance, afforded by large-scale agnostic next-generation sequencing (NGS). An analysis of 180 cisplatin-resistant and cisplatin-sensitive germ cell tumours, in which discovery whole-exome sequencing (WES) in 19 samples and targeted exon-capture-based sequencing of 300 genes in 161 samples was undertaken, was performed. This study confirmed the low mean rate of small mutations (0.9/Mb) with the only frequent recurrent finding in TGCTs being gain of chromosome 12p (REF. 2). Distinguishing features of cisplatin-resistant compared with cisplatin-sensitive tumours were reported: elevated median number of small mutations and enrichment for TP53 mutation or amplifications in the TP53 regulator gene MDM2. Furthermore, WES was performed on 59 tumours from 51 patients with either platinum-sensitive or platinum-resistant tumours, including serial primary and metastatic sites where available. Notably, all TGCTs had wild-type TP53 with expression maintained in both primary and metastatic tumours. This report is the first to show a strikingly high frequency of reciprocal loss-of-heterozygosity copy-number-neutral events in TGCTs, which is much higher than other cancers, and which the researchers replicated in an independent series. The hallmark trio of genomic characteristics in TGCTs were defined as wild-type TP53, chromosome arm 12p gain, and additional reciprocal copy number changes, and similarity to adaptive mutations acquired by human embryonic stem cells was highlighted. The investigators hypothesized that the platinum sensitivity of TGCTs could be associated with high mitochondrial priming and demonstrated an increase in BIM BH3-induced mitochondrial depolarization in TGCTs. Disappointingly, but probably unsurprisingly, these analyses did not converge upon a single neat biomarker of platinum resistance, nor a ready therapeutic target. However, together they offer biological insights into platinum sensitivity; additional depth may be gleaned from broader ‘multiomic’ profiling.

Improved detection and disease monitoring is another clinical priority. Conventional tumour markers (AFP, HCG, and LDH) have been used effectively for early risk stratification and detection of relapse in nonseminomatous GCTs but have limited sensitivity and specificity for patients with seminoma. Circulating microRNAs have had great promise as universal markers for diagnosis and disease monitoring for malignant GCTs since their first description in 2011 (FIG. 1) (REFS 4, 5).

Using the highly sensitive multiplexed pre-amplification qRT–PCR technique, serum levels of a panel of four microRNAs (miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p) were analysed at primary diagnosis of 166 patients with TGCT and 118 men without. Considerably higher serum expression levels for each of the microRNAs was seen in patients with malignant TGCT than in those without. Notably, levels of miR-371a-3p fell to normal after successful completion of treatment, with persistently elevated values in patients who experienced treatment failure and relapse. Using the density estimation model, the sensitivity, specificity, and AUC for miR-371a-3p in the whole cohort were 89%, 93%, and 0.95, respectively, outperforming the conventional serum markers (which have a combined sensitivity of 50%). Analyses of three microRNAs (miR-371a-3p, miR-373-3p, and miR-367-3p) in 250 primary TGCT samples (seminomas and nonseminomatous GCTs) and 164 non-malignant samples similarly showed the sensitivity, specificity, and AUC of these microRNAs combined to be 90%, 91%, and 0.96, respectively; for miR-371a-3p alone these parameters were 90%, 86%, and 0.95, respectively. These studies indicate that miR-371a-3p might ultimately offer the most clinical utility, although

### Key advances

- Large-scale exome sequencing studies of testicular germ cell tumours (TGCTs) revealed distinctive patterns of reciprocal chromosome loss, indicating potential mechanisms underpinning platinum sensitivity
- Large-scale germline exome sequencing of multicase TGCT families revealed a polygenic genomic architecture underlying this disease
- Genome-wide association studies have doubled the number of identified common genetic variants linked to TGCT susceptibility from 25 to 49
- Biomarker studies demonstrated the superiority of a panel of circulating microRNAs (including miR-371a-3p) over traditional serum markers for TGCT diagnosis and monitoring

YEAR IN REVIEW

**TESTICULAR CANCER IN 2017**

**Sequencing advances understanding**

Matthew J. Murray and Clare Turnbull

Our biological understanding of TGCTs has been improved using sequencing, and molecular profiles associated with the genomic evolution and development of cisplatin resistance have been identified. The genomics of variants underpinning TGCT predisposition is being delineated. Studies of circulating microRNAs have demonstrated their potential for noninvasive diagnosis and disease monitoring.

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Epididymis
assembly
factors, and defective microtubule
signalling, transcriptional regulation
• Implicated pathways include
variants
• No major high-penetrance genes
familial relative risk greater (fourfold
• Heritability is higher (49% ) and
Figure 1
NATURE REVIEWS
contribution of rare alleles to TGCT herit -
cases. However, these results suggest that the
required, integrating familial and simplex
case TGCT families
zebrafish model. Mutations in six related
in tumours, as well as in a
ported by demonstration of second-hit muta-
hit was
in the total number of identified TGCT-
GW AS analyses
of the highest effect sizes reported across
studies (GW AS) in TGCT have been remark-
the rationale for assessing the full panel of all
four in prospective trials has been highlighted7.
These two large studies confirm previous reports demonstrating improved sensitivity and specificity of microRNAs compared with conventional serum markers. Validation in prospective clinical trials is under way, her-
alding an opportunity for noninvasive mon-
toring of malignant TGCTs and reduced use of serial CT scans and consequent radiation exposure7 (FIG. 1).

TGCT has a strong heritable basis. In
December 2016, the largest series of germline WES to date, comprising 328 TGCTs from 153 families experiencing multiple instances of
TGCT, was published6. No gene was found in
which disruptive mutations were segregating
in more than 3 of 153 families. The top hit was DNAAF1, but with mutations only
segregating in two families. Implication of
DNAAF1 in TGCT tumorigenesis was sup-
ported by demonstration of second-hit muta-
tions and loss of protein staining for DNAAF1
in tumours, as well as in a DNAAF1hu255h(+/−)
zebrafish model. Mutations in six related
genes were found in 9 of 151 further multi-
case TGCT families8. Larger WES studies are
required, integrating familial and simplex
cases. However, these results suggest that the
contribution of rare alleles to TGCT herit-
ability is highly polygenic. Based on these find-
ings, clinic-based genetic testing for assessing inherited TGCT risk is unlikely to be useful.

Most of the high heritability of TGCT is
likely to be underpinned by common genetic
variants. Results of genome-wide association
studies (GWAS) in TGCT have been remark-
ably fruitful, identifying variants with some of the highest effect sizes reported across
all cancer types. In the past year, two major
GWAS analyses9,10 have resulted in an increase
in the total number of identified TGCT-
associated common variants from 25 to 49. By
genotyping samples from 7,319 men with
TGCTs and 23,082 controls, 19 new TGCT-
associated loci were identified9. Widespread
disruption of developmental transcriptional
regulators, defective microtubule assembly, and
dysregulation of KIT-MAPK signalling
were revealed via chromatin-interaction anal-
ysis as potential mechanisms key in early oncogenesis. A meta-analysis of five
GWAS for TECAC10 identified eight TGCT
susceptibility loci, of which five were novel and
three overlapped with those identified in
the other GWAS9 (FIG. 1).

The growing number of rare and com-
mon susceptibility alleles is complementing
our expanding landscape of molecular deter-
minants of TGCT development and treatment
response. Translation of our advances in mole-
cular understanding of TGCTs into clinical
benefit for patients is the next challenge.

Matthew J. Murray is at the Department of Pathology,
University of Cambridge, Tennis Court Road,
Cambridge CB2 1OP, UK
Clare Turnbull is at the Division of Genetics and
Epidemiology, Institute of Cancer Research,
London SM2 5NG, UK
mjpm16@cam.ac.uk; Clare.Turnbull@icr.ac.uk

Genomic susceptibility
• Heritability is higher (49%) and
familial relative risk greater (fourfold to eightfold) than for other cancers
• Susceptibility to testicular germ cell
tumour (TGCT) is highly polygenic
• No major high-penetration genes
were found using exome sequencing
of families
• Recent genome-wide association
studies identified 49 common
variants
• Implicated pathways include KIT
signalling, transcriptional regulation
of male germ cell development
factors, and defective microtubule
assembly

Somatic profiling of
malignant tumours
• TGCTs have a low rate of somatic
small mutations
• KIT and KRAS are the most frequent
driver genes
• Wild-type TP53 is typically retained
• A large number of copy number and
structural variants are present in
TGCTs
• (11p2) is the hallmark structural
variant of TGCTs
• Features of seminoma: mutant KIT,
hypertriploid
Features of nonseminoma:
• Wild-type KIT, hypotriploid
• Very distinctive patterns of
copy-number-neutral reciprocal
chromosome loss found in TGCTs

Understanding platinum
sensitivity and resistance
• TGCTs are unusually and
exquisitely platinum-sensitive
(overall 5-year survival >95%)
• Mechanisms of platinum
sensitivity are poorly understood
• The TP53 pathway (including
MDM2) is likely to be important
• High mitochondrial priming
could have a role in platinum
sensitivity (as mitochondria have a
high BCL2-related proapoptotic
propensity)

Noninvasive microRNA
monitoring for diagnosis and recurrence
• Current serum tumour markers
(β-fetoprotein, human chorionic
gonadotropin, and lactate dehydroge-
nase) have limited sensitivity and
specificity, particularly for seminomas
• Quantitative real-time PCR of a
panel of four circulating microRNAs
is highly sensitive and specific for
malignant TGCT diagnosis and/or
recurrence monitoring and relapse
detection
• miR-371a-3p is the most individually
predictive microRNA
• MicroRNAs could reduce the
number of CT scans required in
follow-up monitoring
• Prospective clinical trials are
underway

Figure 1 Testicular cancer in 2017 – advances in molecular understanding gained using large-scale sequencing.


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Competing interests statement
The authors declare no competing interests.