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Study information	Outline Form		
Title	TIGER. A randomised phase III trial comparing conventional-Dose chemotherapy using paclitaxel, ifosfamide, and cisplatin (TIP) with high dose chemotherapy using mobilizing paclitaxel followed by High-dose carboplatin and etoposide (TI-CE) as first salvage treatment in relapsed or refractory germ cell tumours		
Study Number	EORTC - 1407 - GUCG	**Leading Group**	Alliance (US-NCI) EORTC coordinates European activities
Study Coordinator	Name: T. Powles	Institution: Barts Health NHS Trust - ST. Bartholomew's Hospital (Number : 558)	
Non-EORTC group(s) involved	Yes ☒ No ☐ Not confirmed ☐	Group(s) name:	Alliance (US-NCI) EORTC will coordinate the participation of European national groups, including France (Unicancer), Germany (KKS), UK (ICR), and Italy.
Sponsorship	EORTC		
Names and contacts of 3 external reviewers *or specify mutual recognition	1 Chris Sweeney (Christopher_Sweeney@DFCI.HARVARD.EDU) 2 Simon Chowdhury (Simon.Chowdhury@gstt.nhs.uk) 3 Peter Grimison (Peter.Grimison@sswahs.nsw.gov.au)		
Concept	Outline Form		
Study background	Germ cell tumors (GCT) are curable diseases. During the past 25 years most clinical problems have been solved and treatment guidelines are universally accepted. Only a small proportion of patients fail to be cured: those who experience a primary resistance to chemotherapy and those who relapsed after first line conventional dose cisplatin-based chemotherapy (CDCT). At present, there is heterogeneity of practice in salvage approaches: to use conventional chemotherapy or to use high dose chemotherapy with autologous stem cell transplant (ASCT). Defining standards and optimizing outcomes of salvage treatment thus represents one of the most pressing issues in GCT treatment at present.		
Rationale and relevance for patients and the scientific community	Due to the lack of randomized trials it remains unclear if CDCT or high dose chemotherapy (HDCT) represents the optimal initial salvage approach for patients with GCT who progressed after first-line chemotherapy. Practices vary throughout the world with some experts (Indiana investigators, German investigators) administering high-dose as initial salvage chemotherapy to all patients whereas others use HDCT only in the third-line setting after failure of initial salvage (2nd-line treatment) with CDCT. Still, others, use a risk stratified approach, with the most favorable patients getting CDCT as initial salvage and the less favorable patients being treated with HDCT. One prior randomized trial (IT-94 study, Pico et al., Ann Onc, 2005, PMID:15928070) attempted to answer this question of the role of high dose therapy in relapsed disease but unfortunately was severely flawed. The study only used 1 cycle of HDCT whereas 2 or 3 cycles of HDCT are considered necessary for optimal benefit. Furthermore, multiple large retrospective studies, including a recent series of nearly 1600 patients, have all suggested a benefit in both PFS and OS for HDCT over CDCT as initial salvage chemotherapy.		

	We believe this represents the most pressing question remaining for defining GCT treatment standards and optimizing outcomes. There are three possible outcomes to this trial, all of which would have important effects on the standard of care for GCT patients: 1. HDCT improves OS compared to CDCT in all risk groups defined by the modified IPFSG scoring system **(see appendix 1)** 2. HDCT does not improve OS compared to CDCT in any risk group. 3. HDCT improves OS relative to CDCT only in a specific risk group. If HDCT offers a true advantage over CDCT in all patient groups (#1 above), then this trial has the potential to both improve the survival of GCT patients and lessen the number of lines of treatment patients will receive in order to achieve cure. All patients (meeting the eligibility criteria of this study) requiring salvage chemotherapy would be routinely recommended for HDCT as initial salvage. As such, many patients would be subject to less overall acute and chronic toxicities, in particular those who would be destined to fail second-line CDCT, only to be cured with third or later-line HDCT. If on the other hand, the trial demonstrates no benefit to HDCT over CDCT as initial salvage therapy (#2 above), then patients will no longer be routinely subjected to HDCT as second-line therapy and this would be reserved only for those patients who do not achieve a cure with second-line therapy. Patients would be spared the burden of routinely being subjected to HDCT if it was unnecessary to achieve cure. Finally, if only certain risk groups of patients benefit from HDCT as initial salvage, then HDCT would be used as initial salvage in only these patients and reserved for third-line treatment in the remaining patients.
Current standard therapy	Conventional dose cisplatin-based chemotherapy (CDCT) represents the current initial salvage approach for patients with GCT who progressed after first-line chemotherapy
Relevant data to justify the use of the control and experimental arms	The treatment arms for this randomized phase 3 trial were carefully selected. For the CDCT arm, TIP was chosen because of the high durable PFS rate (63%) reported in a phase 2 trial of this regimen (Kondagunta et al., JCO, 2005, .PMID: 16170162). As mentioned previously, patient selection certainly contributed considerably to the favorable results reported by Kondagunta et al but there have not been any completed randomized trials comparing TIP to other salvage CDCT regimens. Therefore, it remains unknown whether TIP is superior to any other CDCT regimen. However, it has become a standard salvage treatment at many centers throughout the world and similar efficacy results have never been duplicated with any other salvage CDCT regimen. Therefore, if a trial is to demonstrate superiority of HDCT over CDCT but use a regimen other than TIP, the generalizability of the results might be questioned by skeptics claiming the degree of benefit afforded by HDCT may not have been seen if TIP had been used as the CDCT arm. In selecting the HDCT arm, there were several important considerations. First, it is ideal to have a regimen which has been used to treat all populations that will be studied in the trial. For example, since mediastinal primary tumor patients are to be included in this trial, it would be suboptimal to use a regimen that has not previously been tested in this population. Second, the individual agents used in the HDCT regimen should be as similar as possible to those used in the CDCT regimen but just at lower doses. This limits the variable being tested as much as possible to the intensity of the treatment. For example, if HDCT incorporating paclitaxel were compared with a CDCT regimen not including paclitaxel, it would leave open the possibility that use of paclitaxel could explain a better outcome being observed in the HDCT arm rather than the dose intensity of the chemotherapy. Finally, it is crucial for the HDCT regimen to be highly effective. TI-CE fulfils all of these criteria, since it has been used in patients with tumors of a variety of primary sites with success, it incorporates both ifosfamide and paclitaxel similar to the TIP regimen, and it has demonstrated a high level of effectiveness, despite being targeted to a group with poor prognostic factors. With regards to this last point, the results of a phase I/II trial of TI-CE as salvage therapy was recently reported. The study (Feldman, JCO, 2010, PMID: 20194867) enrolled 107 patients between 1993 and 2006. In order to be eligible for the study, patients had to have at

	least one poor prognostic factor for outcome to salvage CDCT. Poor prognostic factors included 1) Extragonadal primary tumor; 2) Progression after a prior salvage CDCT regimen; 3) Incomplete response or relapse within <6 months after first-line chemotherapy. Of the 107 patients, 21 had mediastinal primary non-seminoma, a group that has historically done quite poorly with both CDCT and HDCT. With a median of 5-years of follow-up, the PFS rate for the entire group was 47% and the OS was 52%. Even patients with mediastinal primary non-seminomas achieved a 24% PFS and 29% OS with TI-CE. These results demonstrate the efficacy of TI-CE HDCT in patients predicted to have poor outcome to CDCT and further support the decision to use TI-CE as the HDCT arm in the current clinical trial.
Patient population (disease characteristics, patient characteristics and prior or concurrent therapy)	**Inclusion criteria** **1. Confirmation of GCT histology** (both seminoma and nonseminoma) on pathologic review at the center of enrollment. Tumor may have originated in any primary site. NOTE: In rare circumstances, patients will be allowed to enroll even if a pathologic diagnosis may not have been established. This would require a clinical situation consistent with the diagnosis of GCT (testicular, retroperitoneal or mediastinal mass, elevated tumor marker levels (HCG $\geq$ 500; AFP $\geq$ 500) and typical pattern of metastases). **2. Must have evidence of progressive or recurrent GCT** (measurable or non-measurable) following one line of cisplatin-based chemotherapy, defined as meeting at least one of the following criteria: a) Tumor biopsy of new or growing or unresectable lesions demonstrating viable non-teratomatous GCT (enrollment on this study for adjuvant treatment after macroscopically complete resection of viable GCT is not allowed). In the event of an incomplete gross resection where viable GCT is found, patients will be considered eligible for the study. b) Elevated serum tumor markers (HCG or AFP) that are increasing. Increase of an elevated LDH alone does not constitute progressive disease. c) Development of new or enlarging lesions in the setting of persistently elevated HCG or AFP, even if the HCG and AFP are not continuing to increase. 3.**Prior treatments** 3.1 **Must have received 3-6 cycles of cisplatin-based chemotherapy as part of first-line (initial) chemotherapy.** Prior POMBACE, CBOP-BEP, or GAMEC are allowed. Note: For patients requiring immediate treatment, 1 cycle of conventional-dose salvage chemotherapy is allowed. Therefore, these patients may have recieved 7 prior cycles of chemotherapy; 6 cycles as part of first-line chemotherapy and 1 cycle of salvage conventional chemotherapy. 3.2 **No more than one prior line of chemotherapy for GCT** (other than the 1 cycle of salvage chemotherapy in Section 3.1) *Definition of one line of chemotherapy:* - *One line of therapy can in some cases consist of 2 different cisplatin-based treatment combinations, provided there is no disease progression between these two regimens. For example, a patient could have received 2 cycles of BEP followed by 2 cycles of VIP if the switch from BEP to VIP was made due to pulmonary toxicity rather than disease progression. This would be considered 1 line of prior therapy. In addition, if a patient received 4 cycles of BEP and then underwent post-chemotherapy resection of residual tumor with findings of residual viable non-teratomatous GCT, and subsequently received 2 additional cycles of adjuvant chemotherapy (EP or an alternate regimen such as VIP) in the absence of disease progression, this would also be considered 1 regimen. However, if any change in therapy is prompted by tumor progression including rising tumor markers, this is considered to represent 2 lines of prior treatment.* - *prior treatment with carboplatin as adjuvant therapy is allowed, provided patients meet other eligibility criteria (e.g., the patient has also received 3-4 cycles of cisplatin-based chemotherapy). Prior treatment with 1-2 cycles of BEP or EP as adjuvant chemotherapy for early stage GCT is allowed, provided the patient also received 3-4 cycles of BEP or EP again at relapse. Patients treated with 3-4 cycles of VIP at relapse following 1-2 cycles of BEP/EP are not eligible as this would be considered more than 1 line of prior therapy.* **3.3 No prior treatment with high-dose chemotherapy** (defined as treatment utilizing stem cell rescue).

3.4 No prior treatment with TIP with the exception when given as a bridge to treatment on protocol for patients with rapidly progressive disease who cannot wait to complete the eligibility screening process. Only one cycle is allowed.
3.5. No concurrent treatment with other cytotoxic drugs or targeted therapies.
3.6 No radiation therapy (other than to the brain) within 14 days of day 1 of protocol chemotherapy except radiation to brain metastases, which must be completed 7 days prior to start of chemotherapy.
3.7 No previous chemotherapy within 17 days prior to enrollment. A minimum of three weeks after the last day of the start of the previous chemotherapy regimen before the first day of chemotherapy on study protocol (e.g., if a patient began their last cycle of BEP on May 1st, they would be eligible for enrollment on May 19th and could begin treatment on May 22nd, even if their last day of treatment was May 5th).
3.8 Must have adequate recovery from prior surgery (e.g., healed scar, resumption of diet, etc.).
4. Age ≥ 14 years (≥ 18 years in Germany)
5. ECOG Performance Status 0 to 2
6. Male gender
7. Laboratory criteria for protocol entry:

a) WBC ≥ 3000/ul or ANC ≥1500/ul (either is sufficient, patients do not need to meet both criteria)
b) Platelets ≥ 100,000/ul
c) Estimated creatinine clearance ≥50mL/min by the Jeliffe equation modified for BSA unless renal dysfunction is due to tumor obstructing the ureters in which case eligibility will be determined by the principal investigator. If the creatinine clearance estimated by the Jeliffe method is ≥50mL/min but ≤70mL/min, then a second method to confirm a creatinine clearance of ≥50mL/min is required. Methods of estimating GFR that can be used for this confirmation consist of a 12 or 24-hour urine creatinine clearance or a nuclear creatinine clearance test. If the confirmatory creatinine clearance is <50mL/min, then the patient is ineligible. If the confirmatory creatinine clearance is ≥50mL/min, the patient is eligible.
d) AST/ALT ≤2.5 x ULN unless due to hepatic metastases in which case levels ≤5xULN are allowed.
e) Bilirubin ≤2 x ULN.

8. No concurrent malignancy other than non-melanoma skin cancer, superficial noninvasive (pTa or pTis) TCC of the bladder, contralateral GCT, or intratubular germ cell neoplasia. Patients with a prior malignancy, but at least 2 years since any evidence of disease are allowed.
9 Negative Serology (antibody test) for the following infectious diseases:
a. Human Immunodeficiency Virus (HIV) type 1 and 2
b. Human T-cell Leukemia Virus (HTLV) type 1 and 2 (mandatory in US but optional in Canada and Europe)
c. Hepatitis B surface antigen
d. Hepatitis C antibody
10 No late relapse with completely surgically resectable disease. Patients with late relapses (defined as relapse ≥ 2 years from the date of completion of the last chemotherapy regimen) whose disease is completely surgically resectable are not eligible. Patients with late relapses who have unresectable disease are eligible.
11 No large (≥ 2 cm) hemorrhagic or symptomatic brain metastases until local treatment has been administered (radiation therapy or surgery). Treatment may begin ≥ 7 days after completion of local treatment. Patients with small (< 2 cm) and asymptomatic brain metastases are allowed and may be treated with radiation therapy and/or surgery concurrently with Arm A or cycles 1 and 2 of Arm B if deemed medically indicated. Radiation therapy should not be given concurrently with high-dose carboplatin or etoposide
12. signed informed consent

Further study guidelines:
Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. Although they will not be considered formal

	eligibility (exclusion) criteria, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol: • Psychiatric illness which would prevent the patient from giving informed consent. • Medical condition such as uncontrolled infection (including HIV), uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient. • Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and are free of disease for ≥ 3 years. • Patients who cannot swallow oral formulations of the agent(s). • Men of reproductive potential should agree to use an appropriate method of birth control throughout their participation in this study due to the teratogenic potential of the therapy utilized in this trial. Appropriate methods of birth control include abstinence, oral contraceptives, implantable hormonal contraceptives or double barrier method (diaphragm plus condom).
Main objective	To compare the overall survival in patients treated with conventional-dose chemotherapy using the TIP regimen (CDCT) with high-dose chemotherapy (HDCT) plus ASCT using the TI-CE regimen as initial salvage treatment of patients with relapsed or refractory GCT.
Secondary objective(s)	1. To compare the progression-free survival (PFS) of patients treated with initial salvage HDCT with TI-CE vs. initial salvage CDCT with TIP. 2. To compare the favorable response rate (FRR) of patients treated with initial salvage HDCT with TI-CE vs. initial salvage CDCT with TIP. 3. To compare the toxicity, including treatment-related mortality, associated with high-dose chemotherapy and ASCT using TI-CE compared with conventional-dose chemotherapy using TIP as initial salvage treatment for patients with relapsed or refractory GCT. 3. To prospectively evaluate the IPFSG scoring system as a predictor of outcome to initial salvage therapy in patients with relapsed or refractory GCT. In this trial, patients will be stratified by a modification of their IPFSG category and we will prospectively evaluate whether or not actual outcomes vary by risk group in the appropriate manner (low risk patients have higher OS than high-risk group). 4. To evaluate the association between tumor marker decline during cycles 1 to 4 with outcome to therapy on either arm.
Study design	This is a randomized (1:1 ratio) phase III trial. Patients will be randomized between 2 arms. Randomization will be stratified by region (North America, Europe) and by modified IPFSG risk classification combining the 5 original groups into 3 risk groups (low, intermediate, and high). The low risk group will consist of very low and low risk patients and the high-risk group will consist of high- and very high-risk patients based on IPFSG criteria.
Integrated biomarker assessment (if not std)	Not applicable
Describe treatment group(s)	Patients will be randomized between: Arm A (TIPx4): Paclitaxel 250mg/m² IV over 24 hours on day 1. Ifosfamide 1500mg/m² (with mesna protection) IV daily from days 2 to 5. Cisplatin 25mg/m² IV daily from days 2 to 5. Neulasta on days 6 or 7 (Neupogen daily from day 7 to 18 or neutrophil recovery). Four cycles, with each cycle administered every 21 days. Arm B (TI-CE): 1) TI: Paclitaxel 200mg/m² IV on day 1. Ifosfamide 2000mg/m² (with mesna protection) IV daily from days 1 to 3. G-CSF 10 micrograms per Kg subcutaneously daily from day 3 until

	day 14 or adequate collection. Leukapheresis starting on day 11. Two cycles of identical therapy will be given 14 days apart. However, if an adequate stem cell collection (≥8 x 106 CD34+ cells/Kg) is achieved with the first cycle, there will be no leukapheresis during the second cycle and the G-CSF dose will be 5 micrograms per Kg daily (instead of 10 micrograms/Kg) from day 4 until adequate neutrophil recovery or day 14. 2) CE: Carboplatin AUC=8 IV daily from days 1 to 3 (equivalent to days -4 to -2 if day 0 is considered the day of stem cell transplant). Etoposide 400mg/m² IV daily from days 1 to 3. Stem cell reinfusion (≥2x106 CD34+ cells/Kg) on day 5. Pegylated G-CSF 6mg subcutaneous on day 5, six hours after stem cell reinfusion. Three cycles of this therapy will be given, each cycle 21 days apart.
Any specific safety issues	Yes, see interim analysis plan
Study scheme	**See study flowchart in appendix 2**
Statistics	Outline Form
Primary endpoint(s) also specify the parameter used in the statistical design	The primary endpoint is overall survival (OS). Overall survival will be defined from the date of randomization to death due to any cause. For surviving patients, OS will be censored on the date the patient was last known to be alive.
Secondary endpoint(s)	1. Progression Free Survival: PFS will be measured from the date of randomization to date of progression or death due to any cause, whichever occurs first. Progression will be defined using the RECIST criteria with tumor markers qualifying as non-target lesions. 2. Favorable Response Rate (FRR): The favorable response rate (FRR) will be defined as the proportion of patients achieving either a complete response (CR) or partial response with normal serum tumor markers (PR-neg) at the time of the end of treatment assessment (see Section 9). Response will be defined as per section 11.0. 3. Treatment-related mortality: Treatment-related mortality will be defined as any death occurring during protocol chemotherapy, or within 30-days following the end of this treatment. 4. Toxicity: All toxicities will be evaluated and recorded based on the NCI common toxicity criteria (CTCAE v4.0). They will be described by frequency and grade, by cycle and over all cycles, with the maximum grade over all cycles used as the summary measure for each patient. 5. Prospective Validation of the IPFG Stratification System (see Tables 1 and 2 below) 6. Biological Correlates
Stats for primary endpoint	Outline Form
Type of study design	Phase III superiority
Is the study randomised	Before start of treatment, 1:1 ratio, stratification for Continent and IPFG risk score using minimization technique (at Alliance center)
Phase III superiority*	null hypothesis including estimate for control group: see below alternative hypothesis as used for the power calculation: see below

	type I and type II errors Alpha 0.05 Beta 0.20 sides 1-sided number of events/ patients 232 events 420 patients expected duration of recruitment 4.2 years expected duration of follow-up after end of accrual 4.5 years
Further details of statistical design	The study is designed such that data from this trial will be combined with data from a similarly designed European trial for a combined analysis. It is anticipated that 168 patients from the Alliance sites and 252 patients from the European sites will be enrolled on the trial. These numbers are approximate and each region will continue to enroll patients even if the target number per site has not been reached. It is expected that a proportion of patients will be cured. The Berkson-Gage exponential cure rate model is used to design this trial. This model assumes that a proportion of patients (p) will be cured and (1-p) who will fail according to an exponential distribution with rate λ. The overall survival function S(t) for the TIP (arm 1) and TICE (arm 2) are expressed as: $S_1(t) = p + (1-p)\exp(-\lambda t)$ $S_2(t) = (p + (1-p)\exp(-\lambda t))^{\theta}$ Respectively, where θ is the hazard ratio under the proportional hazards alternative. It is assumed that 35% of patients will be cured and the median survival time for patients randomized to TIP who are not cured to be 1.5 years. Moreover, it is hypothesized that TICE will reduce the hazard by 29% under that proportional hazards alternative (θ =0.71). The expected information in both arms is 232 deaths. The calculation assume a yearly enrollment rate of 100 patients accrued over 4.2 years and a post-accrual period of 4.5 years after study closure. This design has 81% power assuming a one sided type I error rate of 0.05.
Reference for reference value of design	The reference values for the expectations are taken from the IPFG work, using the expected case mix (% in each risk group) and the expected survival of each risk group, as displayed in the last table of the Appendix 1.
Planned early stopping rule or interim analysis	Efficacy (overall survival) interim analyses for efficacy (RH0) or futility (RH1) will be conducted starting at 25% of the full information (approximately at 30 months after study activation). Other interim analyses will be performed at 55% of the full information (at approximately 48 months after study activation), at 75% (at about 60 months after study activation), at 90% (at about 74 months after study activation), and at 100% (at about 104 months after study activation). Under the alternative hypothesis, 232 deaths are expected at the end of the follow-up period. Critical values at each scheduled analysis will be determined using the Lan-Demets alpha spending function corresponding to the O'Brien-Fleming boundary so that the overall type I error rate of 0.05 is preserved. Should any boundary be crossed, accrual to the study will be stopped. Furthermore, the rate of grade 5 toxicity (NCI-CTC Version 4. criteria) will be monitored and compared between the two treatments arms for the first 200 randomized patients to the study. We expect to accrue the first 200 patients at about 24 months after trial activation. Five interim analyses at 20% (40 patients), 40% (80 patients), 60% (120 patients), 80% (160 patients) and 100% (200 patients) will be performed and discussed at all scheduled conference calls. Assuming a one-sided type I error rate of 0.05, the following Lan-DeMets boundaries will be used for each analysis (–4.23, –2.89, –2.30, –1.96, –1.74). It is assumed that the incidence of unacceptable toxicity in patients treated with TICE is 6%. If at any scheduled time of analysis the lower boundary of a one-sided 90% confidence interval for the difference in unacceptable toxicity exceeds 16%, accrual to the trial will be immediately suspended. In addition, grade 3 and higher toxicity incidence summarized by treatment arm will be presented to the Alliance DSMB for their review.

	Each grade 5 toxicity will be immediately sent by email to the Alliance study team (study chair, statistician and executive officer). The study team will carefully review the toxicities and if any particular site has multiple grade 5 toxicities, then the study team and the Alliance DSMB will consider both the circumstances of the grade 5 toxicities as well as the proportion of patients treated who suffered grade 5 toxicity in deciding whether or not to terminate the study at that institution
Will this trial be monitored by an IDMC	Alliance DSMB since Alliance is the leading group
Central review	Outline Form
Planned central review	Not applicable
Patient Reported Outcomes (PRO), impact assessment, or auxiliary measures	Outline Form
Health Related Quality of Life	Yes, Quality of life will be evaluated using the EORTC QLQ-C30, and the testicular module EORTC QLQ-TC26 at baseline (≤ 21 day prior to registration), end of treatment, month 12, and month 24. Evaluations at the end of treatment, month 12 and month 24 time points may be administered +/- two weeks from the scheduled date.
Health Economics/ Health Technology Assessment	Not applicable
Correlative Translational Research (TR)*	Outline Form
Purpose of correlative TR	Pharmacokinetics / pharmacodynamics
Background and rationale for the TR project (with appropriate literature review and references).	Platinum-based chemotherapy has represented the standard of care for germ cell tumors since its revolutionary introduction against this disease in the 1970s [1]. Despite the fact that cisplatin-based therapy results in cure in most patients, some patients remain refractory or progress. Retreatment with a platinum-including regimen remains the standard for salvage therapy, and the purpose of the larger proposal here is to examine whether a high-dose carboplatin-containing regimen with stem cell support (carboplatin and etoposide, after paclitaxel and ifosfamide stem cell mobilization) is superior to treatment with a conventional dose cisplatin-containing regimen (cisplatin, paclitaxel, and ifosfamide). Beyond the routine use of clinical prognostic scoring systems for patients with germ cell tumors, the prediction of which individuals may benefit from various treatment strategies or regimens does not currently incorporate genetic or pharmacogenetic information. The rapid advances in genome technology over the past decade invite the opportunity to examine such factors within the context of the larger proposed study. Specifically, we propose as a correlative companion study to investigate whether polymorphisms previously associated with platinum response in other disease settings can be informative for the treatment of relapsed/refractory germ cell tumors; and whether novel genetic variants can be identified which may offer prognostic or predictive information in treatment decisions for such patients. We hypothesize that germline SNPs will be identifiable as prognostic disease markers in the entire study population and as predictive markers of treatment benefit in patients receiving platinum-based chemotherapy. The pharmacogenomics of platinum agents has been an area of active interest since discovery of the role of ERCC1, a gene involved in the nucleotide excision DNA repair pathway [2]. ERCC1 has been correlated with both susceptibility to cisplatin toxicity and overall anti-tumor response to cisplatin in various cancers [3], however its role as a germline pharmacogenomic marker has been limited in part because in some diseases tumor tissue is needed to assay ERCC1 mRNA levels in the cancer, and furthermore, germline SNPs in ERCC1 have failed to consistently replicate across studies [4, 5]. ERCC1 represents a candidate gene—a gene known to be involved in the platinum pharmacodynamic pathway. Most pharmacogenetic analyses have taken a candidate gene approach that utilizes biological data to guide the selection of drug response genes in a

pathway. In the case of drug response phenotypes, such candidate gene studies have mostly focused on drug metabolizing enzymes, drug transporters, and genes believed to be involved in the mechanistic pathway of the drug being studied. This approach is limited by our knowledge of the drug phenotype, and thus inherently limits the chance of discovering causal SNPs not involved in mediating drug levels or in a known purported mechanistic pathway [6, 7]. Additionally, it is unlikely that single genes, even candidate genes, entirely explain an individual's drug susceptibility risk [8], meaning that chemotherapeutic sensitivity is likely a multi-genic trait.

Genome-wide approaches permit this possibility and approach identification of pharmacogenomic markers in an unbiased fashion. In contrast to candidate gene studies, genome-wide association studies (GWAS) collect SNP data across the entire human genome and have significant power to detect common variants that confer a modest risk for a complex phenotype [9]. Genome-wide studies capitalize on the large number of SNPs that have been localized and validated across the genome. Whole-genome sequencing takes genome-wide approaches even further and has the ability to interrogate the entire genome, rather than only common SNPs. Technological advances have made genome-wide association studies relatively common and technically easy to perform. Advances in whole-genome sequencing proficiency are similarly making this technology more readily available and affordable quite rapidly.

Two well-performed recent studies have used GWAS approaches to identify novel, interesting variants which may govern response to platinum-based chemotherapy. Both studies included independent replication populations in which testing confirmed a SNP association found in an original discovery set.

The first study [10] identified a novel platinum SNP by first using a previously refined genome-wide discovery approach in cell lines [11, 12]. Utilizing well-genotyped lymphoblastoid cell lines established from healthy individuals in the International HapMap Project [13], carboplatin-specific drug sensitivity phenotypes for multiple cell lines were determined in vitro. Then, GWAS was performed on these lines to associate the chosen phenotype (carboplatin-related sensitivity) with germline SNPs. One of the resulting SNPs (rs1649942) was replicated for association in an independent set of cell lines, and then also replicated clinically by its independent, highly statistically significant association with both PFS and OS (P per-allele = 0.009) in a large study of ovarian cancer patients receiving carboplatin-based chemotherapy [10]. It should be noted that the cell lines used for discovery in this work were from individuals of European descent and the clinical replication population was comprised almost exclusively of individuals of Caucasian ethnicity.

The second study [14] utilized a genome-wide analysis to identify germline SNPs as prognostic factors in small-cell lung cancer patients treated with platinum (either cisplatin or carboplatin) and etoposide. Of 26 SNPs nominally associated in a discovery set of 245 patients, 2 SNPs (rs10895256 and rs716274) were confirmed to be significantly associated with OS in a replication cohort of 305 patients after adjusting for covariates (both $P < 0.002$ after Bonferroni correction) [14]. rs1820453 is of particular interest. Located in the promoter region of YAP1 gene on chromosome 11, a gene encoding a transcriptional activator implicated in P73-dependent apoptosis, the authors found that the T/G polymorphism at rs1820453 forms a transcriptional factor binding site in the promoter of YAP1 , resulting in considerably decreased expression of YAP1 in target lung tissues. The functional significance of the rs1820453 SNP conferring poorer survival could thus be explained by downregulation of YAP1 in patients with the G allele, resulting in suppressed function of P73-dependent apoptosis, and thereby potentially causing poorer responsiveness to chemotherapy-induced apoptotic cell death [14]. While this study was performed in China by including only patients of Han Chinese ethnicity, the identified SNP is prevalent in other ethnic populations. In fact, the HapMap reported minor allele frequency for this SNP in Han Chinese individuals (23%) is similar to that of other world populations (Japanese 20%; Yoruba 25%; CEU Europeans 51%). Testing this SNP in the current proposed international study of germ cell tumor patients would permit potential generalizability of this SNP to a

	more diverse, global population; and would allow potential discernment as to whether this is a prognostic SNP related only to lung cancer prognosis versus potentially a predictive SNP for response to platinum-based therapy. The relatively large size of the current proposed study and the robust response and toxicity phenotype data to be collected make it an ideal sample set both for testing the above two polymorphisms for replication, and for hypothesis-generating whole genome analysis. The identification of SNPs that contribute to response and toxicity of the two regimens being studied will lead to additional studies to understand the mechanism for these associations and to investigate the application of genetic information for the optimization of cancer therapy.
Biosample/ imaging data collection	All participating institutions must ask patients for their consent to participate in the correlative substudies planned for Alliance A031102-PP1, although patient participation is optional. Pharmacogenomic studies and Genomic Wide Associatio Studies will be performed. The pharmacogenetic investigation will take place in germline DNA extracted from a single 10 ml peripheral whole blood specimen sample collected using EDTA vacutainer tubes (lavender tops) prior to beginning the study treatment. Specimen samples will be shipped to the Alliance Biorepository at Ohio State. Genomic DNA will be extracted using a commercially available kit from Qiagen. The concentration and quality of DNA will be quantified by ultraviolet spectroscopy. All DNA specimen samples will be stored in the DNA bank at the OSU Alliance Bank. Aliquots of DNA will be sent to the laboratory responsible for the genotyping. Genotyping for SNPs rs1649942 and rs1820453 will be performed using previously published methods and assay conditions.[24, 41] Consideration will also be given to genome-wide genotyping, with the platform to be determined. This decision will be based on the number of specimen samples collected, the clinical results, and the availability of funding. If genome-wide typing is performed, the results will be deposited into dbGAP, in accordance with NIH policy. Consideration will also be given to exome or whole genome sequencing on some or all of the patients. This decision will be based on the clinical results and the availability of funding. Data deposition will be in accordance with any applicable NIH policy.
Statistical considerations	The primary objective for the proposed pharmacogenomic companion is to validate rs1649942 as a prognostic SNP for progression-free survival (PFS). Specifically, an additive genetic hazards model, with G as the risk allele, is hypothesized The primary analyses will be restricted to the European population. Evidence from a series of GWAS completed by the CALGB suggests that using a combination of self-reported race (white) and ethnicity (non-hispanic) serves as a reasonable surrogate filter to identify a genetic European population. The patient population selection can of course be refined using genome-wide SNP data. This companion will be designed under the assumption that 420 patients will be randomized to the two arms of the clinical study. It is expected that 85% of the patients will provide usable DNA along with consent to the pharmacogenomic analyses and that 85% will self-report as non-hispanic whites. Thus, the expected sample size for the pharmacogenomic analyses will be n=303. The SNP by PFS association will be tested using the Cox score statistic powered for additive risk effects at the one- sided 0.01 level. The assumed relative frequency for the minor allele is 0.24 (Huang et al 2011). The expected event rate, at the time of the analysis, is

	0.55=232/420. For simplicity, we will assume that the time to event distribution in the population is exponential with a median of 2.64 years. The censoring distribution is assumed to be uniform. Under a proportional hazards framework, the minimum genotype hazard ratio (GHR) detectable with a power of 0.83, at the one-sided 0.01 level, is 1.5 (Owzar et al; Gen Epi 2012]. As secondary objectives, we will investigate the association of rs1649942 with overall survival (OS), and rs1820453 with OS and PFS. In addition, we may use the DNA collected to consider other candidate SNPs or to conduct a GWAS to validate other or identify novel candidates, or, as next generation sequencing platforms become more cost effective, consider exome or whole-genome sequencing. The association between germline polymorphisms and other clinical, demographic or molecular (e.g, biomarkers) may also be explored. All SNPs will be evaluated for deviation from Hardy-Weinberg. In the absence of a hypothesized effect, the association analyses will be powered for allele dosing (i.e., additive) effects. To this end, the Cochran-Armitage test (for binary endpoints), Jonkheere-Terpstra test (for quantitative traits including biomarker or gene expressions in serum or tumor RNA) and the Cox score test (for censored time-to-event outcomes) will be used to quantify marginal associations. Multivariable models, with molecular, clinical and demographic variables, will be constructed using conditional inference trees and random forests.
Name of central laboratory and responsible person for biomarker assessment	The samples will be initially stored at the institution and shipped to an EORTC Biobank/biorepository on an annual basis and kept there until batch shipped to the central laboratory.
Name of statistician for TR	Leading group Alliance
Financial support	Movember was preparing a separate grant for this work.
Reference	Outline Form
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Appendix 1: **Modified IPFSG risk classification.**

The prognostic classification was developed by Lorch/Beyer and colleagues and is based on seven individual patient/tumor characteristics at the time of initiation of initial salvage therapy. In this system, each characteristics are associated with a certain point value (ranging from -1 to 3.5 points). The individual characteristics used to calculate the score are:

- **Progression-free interval** >3 months (0 points) vs. ≤3 months (1 point)
- **Response to first-line therapy** of CR/PR-neg (0 points) vs. PR-pos/SD (1 point) vs. PD (2 points)
- **Liver, brain, or bone metastases** absent (0 points) vs. present(1 point)
- **Primary tumor site**: gonadal (0 points) vs. Retroperitoneal (1 point) vs. Mediastinal (3 points)
- **HCG** <1000mIU/mL (0 points) vs. ≥1000mIU/mL (1 point)
- **AFP** normal (0 points) vs. elevated but <1000 (1 point) vs. ≥1000ng/mL (2 points)
- **Histology** of pure seminoma (-1 point) vs. non-seminoma (0 points)

The number of points a patient has for each characteristic are then added together to calculate a final IPFSG prognostic score. Patients are then delineated into 5 risk groups, each with a distinct PFS and OS, based on the value of their score as follows:

- **Very low risk = -1 points**
- **Low risk = 0 points**
- **Intermediate-risk = 1-2 points**
- **High risk = 3-4 points**
- **Very high risk = 5 or more points**

Patients will then be grouped into 3 categories termed Low, Intermediate, and High. The Low risk group will consist of very low and low-risk patients above, and the high-risk group will consist of the high-risk and very high-risk patients. The intermediate-risk group will remain unchanged.

The effect of this on the proportion of patients in each group and the expected 3-year OS taken from the IPFSG paper in JCO is displayed in Table below.

ORIGINAL 5 strata

	% patients	3-year OS %
Very low	13	77
Low	22.6	65.6
Intermediate	37.4	58.3

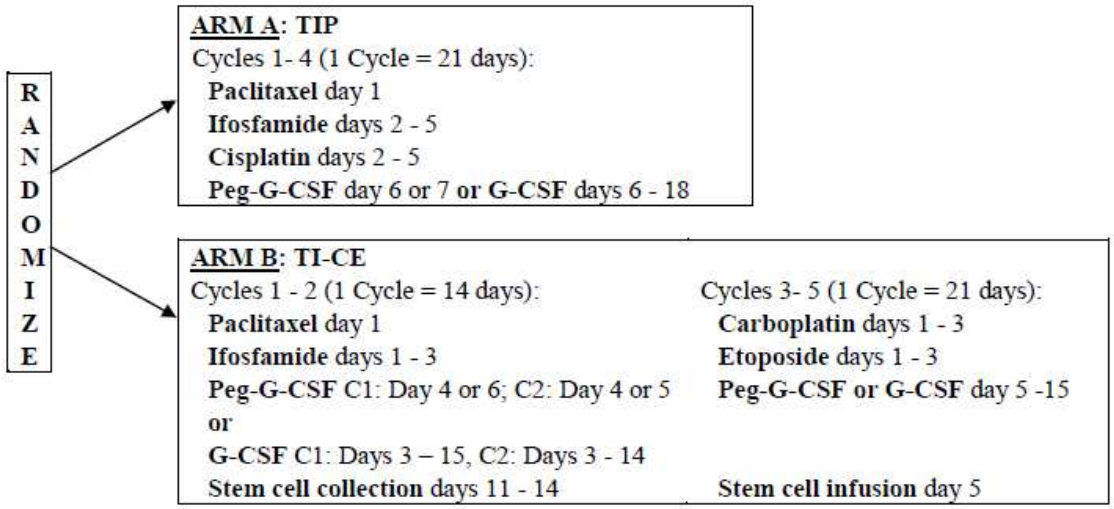
High	20.9	27.1
Very High	6.1	6.1

New COMBINATION 3 STRATA

	% patients	3-year OS
Low (very low + low)	35.6	69.7
Intermediate	37.4	58.3
High (high + very high)	27	22.2

Appendix 2: **Study Flow chart**

Schema



Treatment is to continue until disease progression, unacceptable toxicity, or completion of all protocol treatment, whichever comes first.