

Research Paper

Microsatellite Instability and Loss of Heterozygosity Have Distinct Prognostic Value for Testicular Germ Cell Tumor Recurrence

Alfredo Velasco¹

Erick Riquelme¹

Marcela Schultz²

Ignacio I. Wistuba³

Luis Villarroel¹

Moon S. Koh⁴

Fredrick S. Leach^{4,5,*}

¹Departments of Urology and ²Pathology; Catholic University of Chile; Santiago Chile

³Department of Pathology; MD Anderson Cancer Center; Houston, Texas USA

⁴Department of Urology and ⁵Department of Molecular and Human Genetics; Baylor College of Medicine; Houston, Texas USA

*Correspondence to: Fredrick Leach; Scott Department of Urology; Baylor College of Medicine; Suite 2100; 6560 Fannin Street; Houston, Texas 77030 USA; Tel.: 713.798.3127; Fax: 713.798.5553; Email: fleach@bcm.tmc.edu

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KEY WORDS

genetic instability, germ cell tumor, mismatch repair, testicular cancer

ABBREVIATIONS

MMR	mismatch repair
MSI	microsatellite instability
MSH2	mut-S homolog 2
GCT	germ cell tumor
LOH	loss of heterozygosity

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ABSTRACT

Germ cell tumor (GCT) is the most common genitourinary malignancy of men between the ages of 18 and 35 years. Therapy is ultimately successful in over 90% of patients, however significant morbidity and mortality can be associated with adjuvant treatment and relapse. Molecular markers that predict treatment response and/or poor outcome would have immediate clinical benefit since adjuvant treatment could be selectively reserved for patients at higher risk for relapse and those patients most likely to respond to treatment. In order to identify potential prognostic molecular markers, we evaluated 118 GCT for microsatellite instability (MSI), loss of heterozygosity (LOH) and MSH2 immunostaining to identify tumors associated with relapse and/or poor outcome following initial surgical, medical and/or radiation therapy.

MSI in 3 or more markers and/or low MSH2 staining were associated with relapse while LOH in the absence of MSI and/or high MSH2 staining were not. Twenty-five percent of GCT exhibited genetic instability in 3 or more microsatellite markers (MSI+ tumors), 15% exhibited LOH in the absence of MSI (*LOH only* tumors) and 44% exhibited decreased or absent MSH2 immunostaining (low MSH2 staining tumors). Thirty-six patients (30%) relapsed and 27 of these patients (75%) had MSI+ and/or low MSH2 staining tumors. Only one patient (3%) with an *LOH only* tumor and no patients with high MSH2 staining and *LOH only* tumors relapsed. Therefore distinct GCT subpopulations identified by detection of MSI, LOH and MMR expression are associated with different clinical outcomes. MMR deficient testicular GCT with increased frequency of MSI had an increased association with tumor recurrence compared to GCT with an intact MMR system and LOH in the absence of MSI.

Testicular cancer is the most common genitourinary malignancy in adult men between the ages of 18 and 35 years with 8,980 new cases expected in the United States in 2004.¹ The majority of testicular cancers originate from germ cells and are classified histologically with pure seminoma, embryonal carcinoma, teratoma, choriocarcinoma and mixed histology tumors being the most common subtypes in adolescent and adult males.² Most patients are cured after surgical removal of the affected testicle (radical orchiectomy) with or without adjuvant therapy. However, a relatively constant percentage of affected men will relapse and ultimately succumb to metastatic disease despite aggressive systemic treatment. Primary surgical therapy is curative in over 70% of patients yet adjuvant treatment is offered to most men with clinical stage I (localized) testicular germ cell tumor (GCT) due to the historically high degree of understaging for this malignancy.³⁻⁶

The inability to accurately predict microscopic retroperitoneal disease in men with clinical stage I GCT requires implementation of frequent regimented imaging (surveillance), prophylactic retroperitoneal node dissection (RPLND), adjuvant radiation or chemotherapy. Pathological examination of the primary tumor and measurement of specific tumor markers are helpful but cannot unambiguously define those men destined for relapse following initial treatment for GCT.⁷⁻⁹ As a result, many men undergo unnecessary adjuvant therapy while others destined for relapse choose surveillance to avoid the potential morbidity associated with additional treatment. However, men that elect surveillance potentially lose a disease-free survival benefit from early treatment of clinically non-detectable disease.¹⁰⁻¹³ Molecular and genetic markers that facilitate prognostic assessment by identifying risk factors for tumor recurrence and/or response to primary or salvage therapy would be valuable resources in the clinical management of testicular GCT.

Mismatch repair (MMR) genes encode a family of highly conserved and interacting proteins involved in correcting DNA mispairs formed primarily during DNA replication.¹⁴⁻¹⁶ MMR deficiency leads to genetic instability in hereditary and sporadic human malignancies by enhancing the frequency of cancer promoting mutations found in tumor cell DNA.¹⁷⁻¹⁹ MMR deficiency can be identified by assessing tumor genomic DNA for

increased frequency of replication errors using defined microsatellite repeat sequences.²⁰⁻²² Furthermore, previous investigation of genitourinary malignancies identified MMR deficiency and microsatellite instability (MSI) in testicular GCT and one study suggests prognostic significance with respect to therapeutic resistance.²³⁻²⁵

In order to further investigate the role of genetic instability and MMR expression as prognostic markers in testicular GCT, a panel of ten microsatellite markers and immunostaining for the prototype MMR protein, MSH2, were evaluated in 118 tumors from men diagnosed and treated for this malignancy. We report an association between genetic instability, MSH2 expression and cancer relapse following initial therapy.

MATERIALS AND METHODS

Patients, Clinical and Pathological Characteristics. One hundred eighteen men undergoing radical orchiectomy for germ cell tumor from January 1995 to December 1999 with complete clinical follow up for at least five years were included in this study. Clinical stage refers to the stage at initial diagnosis. The average age of the patients at initial diagnosis was 25 years (range 16–45). The predominant tumor histology within the radical orchiectomy specimen determined the subtype and included pure seminoma, embryonal carcinoma, teratoma, choriocarcinoma and mixed histology tumors. Clinical and pathological stage was according to the American Joint Commission on Cancer (AJCC) as summarized in the appendix.

Initial therapy was defined as the treatment carried out at the time of initial diagnosis and included radical orchiectomy for all patients followed by surveillance, RPLND, chemotherapy, or radiation therapy depending on clinical stage at presentation, tumor histology and pathological findings. Tumor recurrence or clinical relapse was defined as patients with newly diagnosed retroperitoneal lymphadenopathy or extra-lymphatic disease after completing primary surgical and (if indicated) radiation or medical therapy for clinically localized (stage I) or advanced (stage IIB or higher) disease. For pure seminoma, all men with clinical stage I disease received adjuvant radiation therapy. For non-seminoma, men with clinical stage I disease were given the option of surveillance, RPLND or adjuvant chemotherapy consisting of bleomycin, etoposide and cisplatin (BEP). All men with advanced disease (including those with microscopic metastasis discovered after RPLND for stage I disease) received 3–6 rounds of BEP chemotherapy following orchiectomy regardless of histological subtype.

Microsatellite and Immunohistochemical Analyses. Adjacent normal and tumor archival tissues were obtained from the Pathological Anatomy Department of the Catholic University of Chile and used for genetic and immunohistochemical analyses. Genetic instability was identified after comparing genomic DNA from matched normal and tumor tissues as previously described.²⁶ Microsatellite markers used for analysis were: D2S123, D3S1283, D3S1293, D3S1029, D9S66, D9S113, TP53CA, LNSCA, BAT 25 and BAT 26 (Research Genetics, Invitrogen Life Technology). Immunohistochemical analysis used a previously described and characterized monoclonal antibody specific for the MSH2 protein.^{26,27} In all cases, the molecular and genetic findings were with respect to the primary testicular germ cell tumor.

Statistical Analysis. Fisher's exact tests determined statistical significance as defined by p values <0.05. For cases where the distribution was significantly asymmetrical, non-parametrical systems were also utilized. In some

Table 1 **HISTOLOGY, CLINICAL STAGE AND OUTCOME**

No. (%)	All Tumors N = 118 (100)	Seminoma N = 44 (37)	Embryonal N = 42 (36)	Mixed N = 25 (21)	Teratoma N = 5 (4)	Chorio. N = 2 (2)
Stage						
Localized	86 (73)	37 (84)	30 (71)	15 (60)	2 (40)	2 (100)
Advanced	32 (27)	7 (16)	12 (29)	10 (40)	3 (60)	0 (0)
Recurrence						
No	82 (70)	29 (66)	29 (69)	20 (80)	3 (60)	1 (50)
Yes	36 (30)	15 (34)	13 (31)	5 (20)	2 (40)	1 (50)
Outcome						
Alive	108 (92)	42 (95)	37 (88)	23 (92)	4 (80)	2 (100)
Dead	10 (8)	2 (5)	5 (12)	2 (8)	1 (20)	0 (0)

Chorio, choriocarcinoma.

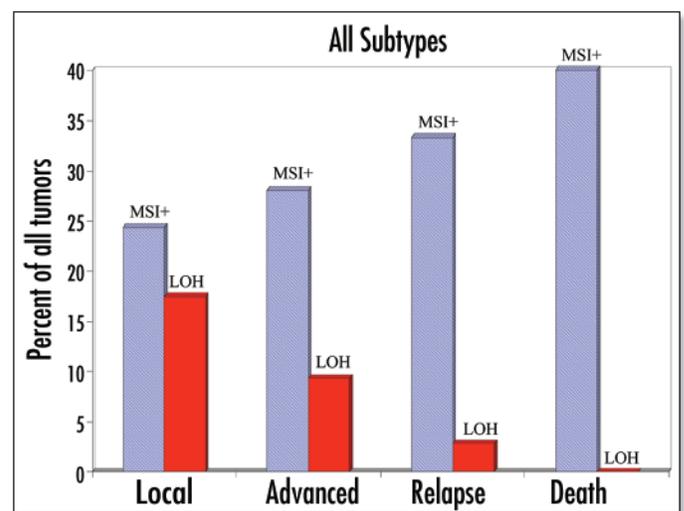


Figure 1. Analysis of genetic instability in testicular GCT as percent of all tumors with respect to clinical stage (local or advanced) and outcome (relapse and death). Hatched bars, MSI+ tumors; solid bars, LOH only tumors. MSI+, microsatellite instability in 3 or more markers; LOH, loss of heterozygosity in the absence of MSI.

cases the numerical data was rounded to the nearest whole number prior to statistical analysis.

RESULTS

Clinical and Pathological Characteristics. The histological subtype, frequency and clinical characteristics for the 118 GCT used in this study are shown in Table 1. Eighty-six men (73%) had localized and 32 men (27%) had advanced disease. Pure seminoma was the most frequent GCT subtype (N = 44) and 84% were localized (clinical stage I). Embryonal carcinoma (N = 42) and mixed histology tumors (N = 25) were localized in 71 and 60% of the patients respectively while teratoma (N = 5) and choriocarcinoma (N = 2) were localized in 40 and 100% of cases respectively.

Tumor recurrence was diagnosed in 36 patients (30%) following initial therapy and 15 patients (34%) with pure seminoma experienced a clinical relapse (Table 1). Thirteen patients (31%) diagnosed with embryonal carcinoma and five patients (20%) diagnosed with mixed histology tumors relapsed while three of the seven patients diagnosed with either teratoma or choriocarcinoma relapsed (Table 1). Ten patients (8%) died of treatment refractory GCT and one patient died from trauma not related to his malignancy (Table 1). Five deaths were due to treatment refractory embryonal

Table 2 GENETIC INSTABILITY, CLINICAL STAGE AND OUTCOME

No. (%)	All Tumors N = 118 (100)	MSI+ N = 30 (25)	LOH Only N = 18 (15)	p-value
Stage				
Localized	86 (73)	21 (70)	15 (83)	NS
Advanced	32 (27)	9 (30)	3 (17)	
Recurrence				
No	82 (70)	18 (60)	17 (94)	0.017
Yes	36 (30)	12 (40)	1 (6)	
Outcome				
Alive	108 (92)	26 (87)	18 (100)	NS
Dead	10 (8)	4 (13)	0 (0)	

NS, not statistically significant.

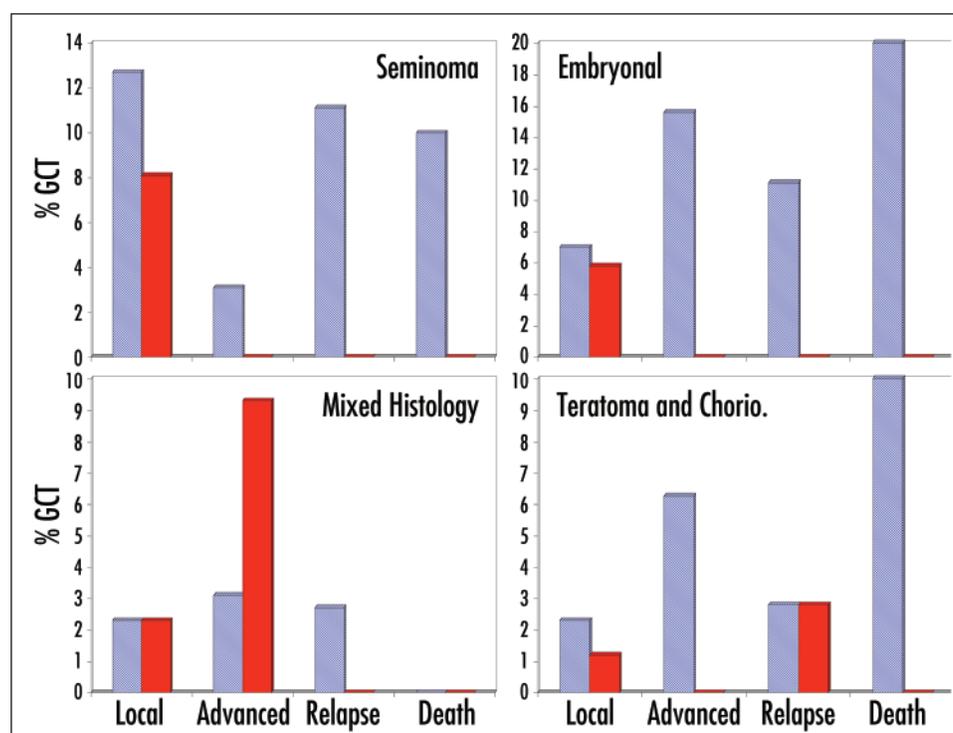


Figure 2: Analysis of genetic instability in testicular GCT as percent of all tumors by histological subtype and with respect to clinical stage (local or advanced) and outcome (relapse and death). Hatched bars, MSI+ tumors; solid bars LOH only tumors. Chorio, choriocarcinoma.

carcinoma and two deaths resulted from treatment refractory seminoma. Two patients died as a result of treatment refractory mixed histology tumors and one patient from treatment refractory teratoma.

Genetic Instability, Clinical Stage and Outcome. We previously identified distinct testicular GCT subpopulations as defined by MSI in three or more markers (MSI+ tumors) or LOH in the absence of MSI (LOH only tumors).²⁶ MSI+ tumors represented 24% of localized and 28% of advanced stage GCT while LOH only tumors represented 17% of localized and 9.3% of advanced stage GCT (Fig. 1). MSI+ tumors represented 33% of the patients experiencing relapse while LOH only tumors represented less than 3% of these patients (Fig. 1). MSI+ tumors represented 40% of patients that died from treatment refractory GCT while LOH only tumors were not detected in patients that ultimately died from treatment refractory GCT (Fig. 1). As a

group, 70% of MSI+ and 83% of LOH only tumors were localized (Table 2). Twelve patients (40%) with MSI+ tumors recurred compared to only one patient (6%) with an LOH only tumor (Table 2). Four patients (13%) with MSI+ tumors died from treatment refractory GCT compared to no patient deaths in the LOH only tumor group (Table 2).

Next, specific histological subtypes were assessed with respect to genetic instability, clinical stage and outcome. MSI+ pure seminoma represented 12.7% of all localized GCT while MSI+ embryonal carcinoma, MSI+ mixed histology tumors and MSI+ teratoma combined with choriocarcinoma represented 7, 2.3 and 2.3% of all localized GCT respectively (Fig. 2). MSI+ embryonal carcinoma represented 15.6% of all advanced stage GCT while MSI+ teratoma combined with choriocarcinoma, MSI+ seminoma and MSI+ mixed histology tumors represented 6.2, 3.1 and 3.1% of advanced stage GCT respectively (Fig. 2). When considering patients experiencing clinical relapse, MSI+ pure seminoma and MSI+ embryonal carcinoma each represented 11.1% of GCT failing initial therapy while MSI+ mixed histology tumors and MSI+ teratoma combined with choriocarcinoma represented 2.7 and 2.8% of tumors failing initial therapy respectively (Fig. 2). MSI+ embryonal carcinoma represented 20% of patient deaths due to treatment refractory GCT while MSI+ pure seminoma and MSI+ teratoma combined with choriocarcinoma each represented 10% of patient deaths (Fig. 2). No patients with MSI+ mixed histology tumors died from treatment refractory GCT.

LOH only pure seminoma represented 8.1% of localized GCT while LOH only embryonal carcinoma, LOH only mixed histology tumors and LOH only teratoma combined with choriocarcinoma represented 5.8, 2.3 and 1.2% of all localized GCT (Fig. 2). LOH only mixed histology tumors represented the 9.3% of advanced stage GCT while LOH only teratoma combined with choriocarcinoma represented the 2.8% of GCT associated with clinical relapse following initial therapy (Figs. 1 and 2). LOH only pure seminoma and LOH only embryonal carcinoma were not detected in advanced clinical stage GCT or patients experiencing clinical relapse (Fig. 2).

MSH2 Expression, Clinical Stage and Outcome. Low or high MSH2 immunostaining was found in 52 (44%) and 66 (56%) respectively of all GCT used in this study (Table 3). When considering low MSH2 staining tumors, 29 (56%) were localized, 23 (44%) advanced, 25 (48%) recurrent and 6 (12%) detected in patients that died from treatment refractory GCT (Table 3). For high MSH2 staining tumors, 59 (89%) were localized, 7 (11%) advanced, 11 (27%) recurrent and 4 (6%) detected in patients

that died from treatment refractory GCT (Table 3). When considering clinical stage, low MSH2 staining tumors represented 34 and 72% of localized and advanced stage GCT respectively while high MSH2 staining tumors represented 66 and 28% of localized and advanced stage GCT respectively (Fig. 3). When considering tumor recurrence, 69% of patients experiencing clinical relapse had low MSH2 staining tumors compared to 31% of patients with high MSH2 staining tumors (Fig.3). Sixty percent of patients that died from treatment refractory GCT had low MSH2 staining tumors and 40% had high MSH2 staining tumors (Fig. 3).

Next, we compared degree of MSH2 immunostaining in specific histological subtypes with respect to clinical stage and outcome. Low and high MSH2 staining pure seminoma represented 7 and 36% of localized GCT respectively while low and high MSH2 staining embryonal carcinoma

Table 3 **MSH2 EXPRESSION, CLINICAL STAGE AND OUTCOME**

No. (%)	All Tumors N = 118 (100)	MSH2 Low N = 52 (44)	MSH2 High N = 66 (56)	p-value
Stage				
Localized	86 (73)	29 (56)	59 (89)	<0.0001
Advanced	32 (27)	23 (44)	7 (11)	
Recurrence				
No	82 (70)	27 (52)	55 (83)	0.0003
Yes	36 (30)	25 (48)	11 (27)	
Outcome				
Alive	108 (92)	46 (88)	62 (94)	NS
Dead	10 (8)	6 (12)	4 (6)	

NS, not statistically significant.

represented 16.3 and 18.6% of localized GCT respectively (Fig. 4). Low and high MSH2 staining mixed histology tumors represented 7 and 10.5% of localized GCT respectively while low and high MSH2 staining teratoma combined with choriocarcinoma represented 3.5 and 1.2% of localized GCT respectively (Fig. 4). Low and high MSH2 staining pure seminoma represented 18.8 and 3.1% of advanced stage GCT respectively while low and high MSH2 staining embryonal carcinoma represented 25 and 12.5% of advanced stage GCT respectively (Fig. 4). Low and high staining mixed histology tumors represented 18.8 and 12.5% of advanced stage GCT while low and high MSH2 staining teratoma combined with choriocarcinoma represented 9.4 and 0% of advanced stage GCT respectively (Fig. 4).

Low and high MSH2 staining pure seminoma represented 30.6 and 11.1% of GCT from patients experiencing clinical relapse following initial therapy respectively while low and high MSH2 staining embryonal carcinoma represented 22.2 and 13.9% of GCT from these patients respectively (Fig. 4). Low and high MSH2 staining mixed histology tumors represented 8.3 and 5.6% of GCT from patients experiencing clinical relapse respectively while

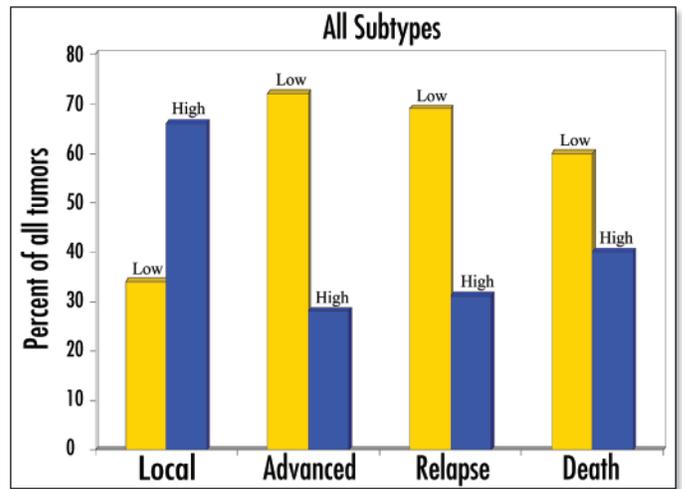


Figure 3: Analysis of MSH2 immunostaining in testicular GCT as percent of all tumors with respect to clinical stage (local or advanced) and outcome (relapse and death). Light bars, low MSH2 staining tumors; dark bars, high MSH2 staining. High, high MSH2 staining; Low, low MSH2 staining.

low and high MSH2 staining teratoma combined with choriocarcinoma represented 8.3 and 0% of GCT from patients experiencing relapse respectively (Fig. 4). Low or high MSH2 staining embryonal carcinoma represented 20 and 30% of tumors from patients that died from treatment refractory GCT respectively while low or high MSH2 staining mixed histology tumors each represented 10% of GCT from these patients (Fig. 4). Low MSH2 staining pure seminoma and low MSH2 staining teratoma combined with choriocarcinoma represented 20 and 10% of tumors from patients that died from treatment refractory GCT respectively (Fig. 4). No high MSH2 staining pure seminoma or high MSH2 staining teratoma combined with choriocarcinoma were associated with patient deaths due to treatment refractory GCT (Fig. 4).

Genetic Instability, MSH2 Expression, Clinical Stage and Outcome. Low MSH2 staining MSI+ (low/MSI+) and high MSH2 staining LOH only (high/LOH) testicular GCT were compared with respect to clinical stage and outcome. Low/MSI+ tumors (N = 22) represented 19% and high/LOH tumors (N = 14) represented 12% of all testicular GCT used in this study (Table 4). Low/MSI+ and high/LOH tumors represented 13.9 and 15.1% of all localized GCT respectively (Fig. 5). In contrast, low/MSI+ tumors represented 28% of advanced stage GCT while high/LOH tumors represented 3.1% of advanced stage GCT (Fig. 5). When considering tumor recurrence, low/MSI+ tumors were detected in 27.8% of patients failing initial therapy while high/LOH tumors were not detected in patients experiencing clinical

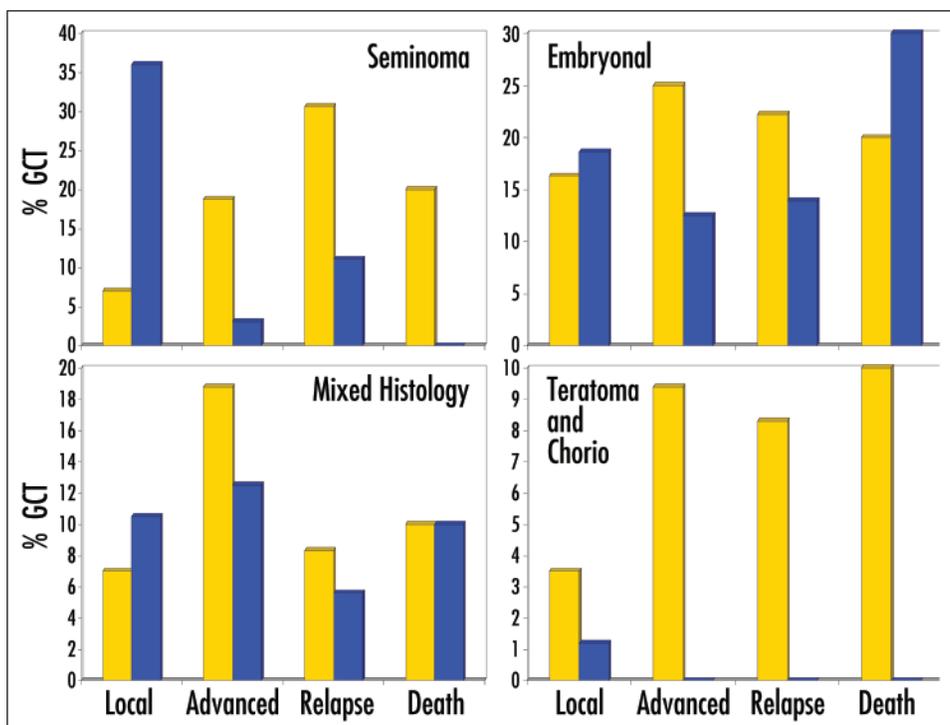


Figure 4. Analysis of MSH2 immunostaining in testicular GCT as percent of all tumors by histological subtype and with respect to clinical stage (local or advanced) and outcome (relapse and death). Light bars, low MSH2 staining tumors; dark bars, high MSH2 staining. Chorio., choriocarcinoma.

Table 4 **GENETIC INSTABILITY, MSH2 EXPRESSION, CLINICAL STAGE AND OUTCOME**

	All Tumors N = 118 (100)	Low/MSI+ N = 22 (19)	High/LOH N = 14 (12)	p-value
Stage				
Localized	86 (73)	13 (59)	13 (83)	0.054
Advanced	32 (27)	9 (41)	1 (17)	
Recurrence				
No	82 (70)	12 (54)	14 (100)	0.0027
Yes	36 (30)	10 (46)	0 (0)	
Outcome				
Alive	108 (92)	18 (82)	14 (100)	NS
Dead	10 (8)	4 (18)	0 (0)	

NS, not statistically significant.

relapse (Fig. 5). Low/MSI+ tumors were detected in 40% of patients that died from treatment refractory GCT while no high/LOH tumors were detected in patients that ultimately died after initial therapy (Fig. 5).

As a group, 59% of low/MSI+ and 83% of high/LOH GCT were clinically localized respectively (Table 4). Forty-six percent of low/MSI+ tumors were detected in patients experiencing clinical relapse and 18% of low/MSI+ tumors were associated with patient death due to treatment resistant GCT (Table 4). Overall, 27 of the 36 patients (75%) experiencing clinical relapse had either MSI+, low MSH2 staining or low MSH2 staining MSI+ tumors (Tables 2–4). High/LOH tumors were not detected in patients that relapsed or died following initial or salvage therapy for testicular GCT (Fig. 5).

When assessing genetic instability and degree of MSH2 immunostaining by histological subtype, low/MSI+ pure seminoma represented 4.6 and 3.1% of localized and advanced stage GCT respectively while low/MSI+ embryonal carcinoma represented 5.8 and 15.6% of localized and advanced stage GCT respectively (Fig. 6). Low/MSI+ mixed histology tumors represented 2.3

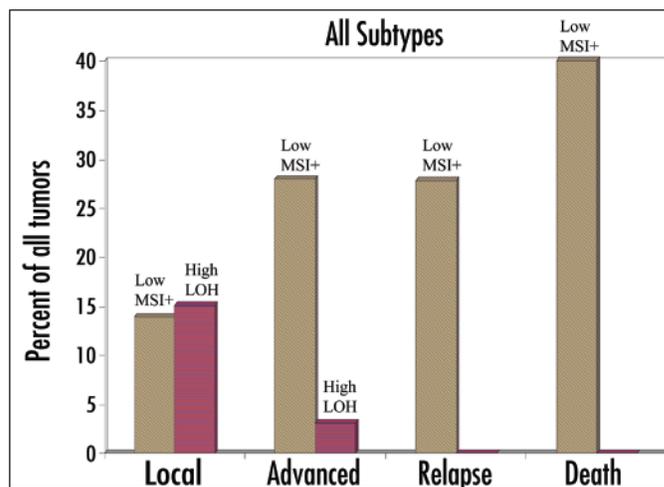
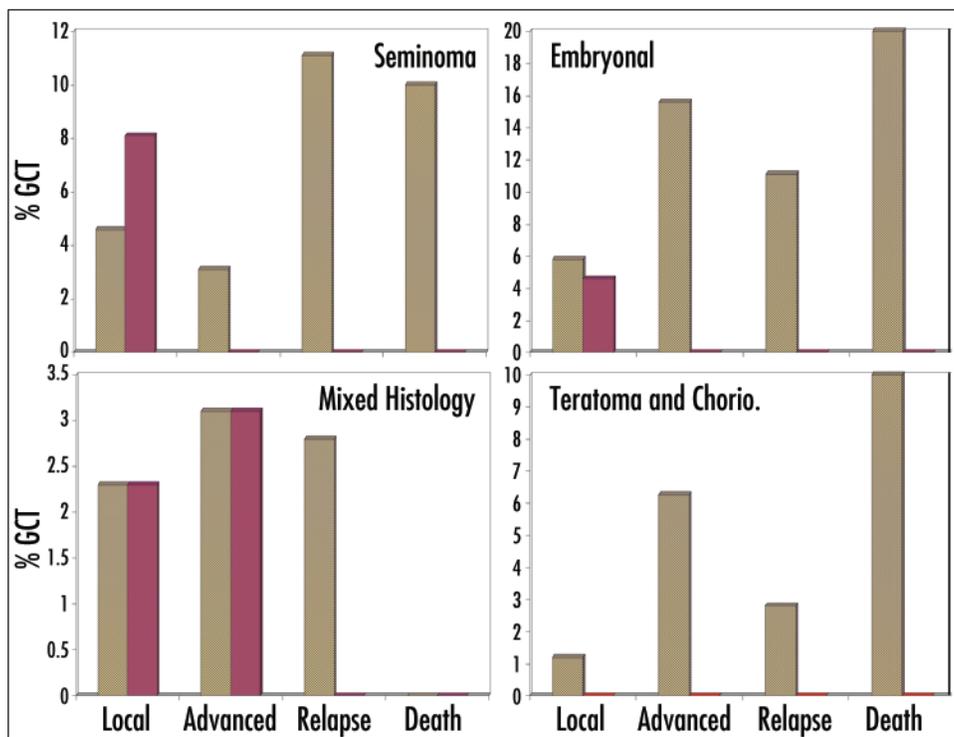


Figure 5. Analysis of genetic instability and MSH2 immunostaining in testicular GCT as percent of all tumors with respect to clinical stage (local or advanced) and outcome (relapse and death). Diagonal hatched light bars, low MSH2 staining MSI+ tumors; horizontal hatched dark bars, high MSH2 staining LOH only tumors.

and 3.1% of localized and advanced stage GCT respectively while low/MSI+ teratoma combined with choriocarcinoma represented 1.2 and 6.2% of localized and advanced stage GCT respectively (Fig. 6). Low/MSI+ pure seminoma represented 11.1% of GCT detected in patients experiencing clinical relapse and 10% of patient deaths from treatment refractory GCT (Fig. 6). Low/MSI+ embryonal carcinoma represented 11.1% of GCT detected in patients experiencing clinical relapse and 20% of patient deaths from treatment refractory GCT (Fig. 6). Low/MSI+ mixed histology tumors represented 2.8% of GCT from patients experiencing clinical relapse and no patient deaths from treatment refractory GCT (Fig. 6). Low/MSI+ teratoma combined with choriocarcinoma represented 2.8% of tumors detected in patients experiencing clinical relapse and 10% of patient deaths due to treatment refractory GCT (Fig. 6).



High/LOH pure seminoma represented 8.1% of localized GCT while high/LOH embryonal, high/LOH mixed histology tumors and high/LOH teratoma combined with choriocarcinoma represented 4.6, 2.3 and 0% of localized GCT respectively (Fig. 6). High/LOH mixed histology tumors represented the 3.1% of advanced stage GCT since no other high/LOH histological subtypes were detected in advanced stage GCT (Figs. 5 and 6). As above, no high/LOH tumors were detected in patients that experienced clinical relapse or death following initial or salvage therapy (Figs. 5 and 6). The statistical significance of genetic instability, degree of MSH2 staining or genetic instability and degree of MSH2 staining with respect to clinical stage and outcome are shown in Tables 2–4.

Figure 6. Analysis of genetic instability and MSH2 immunostaining in testicular GCT as percent of all tumors by histological subtype and with respect to clinical stage (local or advanced) and outcome (relapse and death). Diagonal hatched light bars, low MSH2 staining MSI+ tumors; horizontal hatched dark bars, high MSH2 staining LOH only tumors. Chorio, choriocarcinoma.

DISCUSSION

We investigated the clinical relevance of genetic instability and MMR expression in testicular GCT using a panel of microsatellite markers and MSH2 immunostaining. Clinical response following initial therapy was evaluated by comparing MSI, LOH and degree of MSH2 staining to clinical stage and outcome. Our results demonstrated a correlation between MSI+ and/or low MSH2 staining tumors with clinical relapse following initial therapy when compared to *LOH only* tumors ($p = 0.017$), high MSH2 staining tumors ($p = 0.0003$) or high MSH2 staining and *LOH only* tumors ($p = 0.0027$). Low MSH2 staining was also associated with advanced stage compared to high MSH2 staining tumors ($p < 0.0001$). We previously identified an association between reduced MMR expression and genetic instability in testicular GCT²⁶ and our current work supports a hypothesis that these two molecularly distinct GCT subpopulations have different clinical outcomes following standard initial treatment.

Previous investigation of MMR in testicular GCT and other tumors suggested that genetic instability in the form of MSI may contribute to treatment failures by facilitating tumor resistance.^{23,28} In a previous study of testicular GCT, Mayer et al found that MSI+ tumors were associated with chemotherapeutic resistance.²³ Our study generally supports and expands upon their findings by distinguishing tumors with LOH in the absence of MSI as a separate testicular GCT subgroup with a distinct prognostic profile. Our findings were consistent within histological subtypes suggesting that molecular assessment of testicular GCT can complement standard pathological analysis with respect to clinical prognosis. Finally, our results provide a molecular and genetic framework for designing and implementing clinical trials for the rationale use of adjuvant therapy in men with GCT and perhaps other malignancies.

The molecular and clinical findings in testicular GCT appear to contradict previous findings in prostate cancer where MMR deficiency predicted a more favorable prognosis with respect to biochemical recurrence.^{29,30} However, this difference may not be surprising when considering the different clinical context and tumor biology of these two male specific malignancies. In patients treated surgically for clinical stage I adenocarcinoma of the prostate, additional therapy is reserved for clinical or pathological evidence of advanced stage or disease progression. In contrast, patients treated surgically for clinical stage I testicular GCT are given additional therapy based on historical precedent and histopathological findings in the primary tumor. As a result, the vast majority of men in this study received either adjuvant radiation or chemotherapy. Therefore we cannot correlate our molecular findings with the natural progression of clinically localized GCT as in our previous analysis using patients with clinically localized prostate cancer undergoing prostatectomy as the only initial therapy. Alternatively, MMR deficiency in testicular GCT may have fundamentally different biological and clinical consequences compared to MMR deficiency in prostate cancer regardless of initial or subsequent therapy. In any case, future investigation of these two male specific malignancies may provide important insights into the biological and clinical consequences of MMR deficiency.

Appendix: AJCC Staging for Testicular GCT

Clinical stage I: No evidence of regional lymph node enlargement or distant metastasis.

Clinical stage IIa: Regional lymph node enlargement 2 cm or less and no distant metastasis.

Clinical stage IIb: Regional lymph node enlargement greater than 2 cm but not greater than 5 cm and no distant metastasis.

Clinical stage IIc: Regional lymph node enlargement greater than 5 cm and no distant metastasis.

Clinical stage III: Non-regional nodal, pulmonary or visceral metastasis.

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