

The role of microsatellite instability, mismatch repair deficiency and *BRAF* mutation in chemotherapy resistance of germ cell tumors

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In Western countries, testicular germ cell tumors (GCTs) of adolescents and adults, the so called type II TGCTs are the most frequent solid cancer found in Caucasian men aged 20-45 years accounting for up to 60% of all malignancies in this group [1]. The incidence is increasing continuously over the last decades. Due to a unique sensitivity to cisplatin-based chemotherapy, up to 80% of patients with metastatic disease can be cured by combination of chemotherapy and tumor resection in case of residual disease [2]. However, even with modern treatment, type II GCTs are a deadly disease in a minority of cases (approximately 5% of all patients), and the biology underlying treatment failure is poorly understood [3]. Various findings point towards a key role of DNA damage response in the exceptional cure rates in these cancers achieved by DNA damaging therapy [4]. The role of mismatch repair (MMR) deficiency and microsatellite instability (MSI) has previously been investigated, yet results have been controversial [5-8]. A correlation between MSI and activating mutations like *BRAF* V600E, as has been described in colorectal cancer [9-11], has not been investigated in resistant type II GCTs so far.

In the Laboratory of Experimental Patho-Oncology (ErasmusMC, Rotterdam, the Netherlands, headed by prof., dr. Leendert H.J. Looijenga) I was able to analyze a unique series of 35 clinically well documented chemotherapy resistant type II GCTs and to compare them with a series of 100 controls. We can show for the first time a correlation between a gene mutation – *BRAF* V600E – and cisplatin resistance in non-seminomatous type II GCTs. Furthermore a correlation between MMR

deficiency, MSI and treatment failure is confirmed from studies done in this workgroup before [3, 6].

MSI was examined in 135 type II GCTs and corresponding normal tissue using a panel of established mono- and dinucleotide markers, including the so called “Bethesda panel” [12] (BAT25, BAT26 as mono-, and D2S123, D5S346, D17S250, BATRII, BAT40, and MSH6 as dinucleotide markers). In the cohort of 35 resistant tumors we could find an incidence of MSI affecting a single locus in two cases (6%, designated MSI low), and in nine cases showing two or more affected loci (26%, designated MSI high). The most frequently affected loci were D2S123, D5S346, D17S250, and BAT40, whereas MSH6, BAT25 and BAT26 were never affected. In the cohort of 100 controls, six tumors (three seminomas, three non-seminomas) showed MSI (6% in total). All cases were MSI low, affecting exclusively dinucleotide markers: BAT40 in the three non-seminomas, and D17S250, BAT40, and MSH6 in one case each of the seminomas. The difference in MSI high between unselected and refractory GCTs was statistically significant ($p < 0.001$). Clinically the MSI high cases showed no difference in overall survival (34 months versus 18 months, $p = 0.37$), but a trend towards a longer median progression-free survival (12 months versus 6 months, $p = 0.068$), compared to the MSI low and microsatellite stable (MSS) cases.

The incidence of *BRAF* mutation 1796T>A (resulting in V600E) in both resistant and unselected tumors was assessed using the high sensitive FRET technique. Nine resistant non-seminomas (26%) showed a *BRAF* mutation, whereas only one tumor (non-seminoma) in the group of 100 unselected patients was mutated. This difference between the groups was highly significant ($p < 0.001$). The *BRAF* mutated cases showed no differences in overall survival (34 months versus 18

months, $p=0.34$) or median progression-free survival (12 months versus 6 months, $p=0.19$) compared to patients with wild-type *BRAF*.

In the control series, expression of hMLH1, hMLH2, hMSH2 and PMS2 by immunohistochemistry was found in seminomas ($n=50$) in 98%, 98%, 98%, and 50% and in non-seminomas ($n=50$) in 96%, 98%, 98%, and 48%, respectively. In the series of resistant tumors, assessment of MMR proteins was possible in 33 and 34 cases for hMLH1/PMS2 and hMSH2/hMSH6 respectively. Expression of hMLH1, hMLH2, hMSH2 and PMS2 was found in 76%, 91%, 88%, and 30%, respectively. The percentage of tumors showing no or low expression of either hMLH1 or hMSH6 was significantly higher in the resistant samples, compared to the unselected tumors ($p=0.001$ and 0.036 , respectively, Fisher's Exact Test). In the group of the resistant tumors, *hMLH1* promotor methylation was detected in nine tumors (31%). Methylation correlated highly with lack of hMLH1 protein expression ($p<0.001$, Pearson correlation). Weak or absent immunohistochemical staining of hMLH1 correlated with both MSI and mutated *BRAF* ($p=0.017$ and $p=0.008$, Pearson correlation). Furthermore, weak or absent PMS2 staining correlated with mutated *BRAF* ($p=0.04$). In tumors showing MMR deficiency, positive correlations were found between low or absent expression of hMLH1/PMS2 (so called hMutL α complex, $p=0.02$) and hMSH2/hMSH6 (so called hMutS α complex, $p<0.001$).

To my knowledge, this present series of 35 truly resistant cases is the largest ever analyzed in GCTs with respect to molecular markers. Assuming that approximately 90-95% of all GCT patients (all histologies, all stages) will achieve long term survival [13], this series represents a subgroup suffering from treatment failure of an estimated total of 400-700 GCT patients. Having discovered a correlation between MSI and chemotherapy resistance in GCTs, our findings were confirmed by others [7, 8, 14] and our analyses reveal a critical role of both MMR deficiency and

MSI, and for the first time describe a correlation of *BRAF* mutation V600E with treatment resistance in non-seminomatous GCTs. In contrast to differences depending on histological elements our data suggest the existence of a distinct molecular subtype of GCTs, characterized by cisplatin resistance, MSI high, low or absent expression of MMR proteins, at least partially caused by promoter hypermethylation, and high incidence of *BRAF* mutation V600E. In fact, no significant differences in molecular markers or clinical response between the histological subgroups have been found and no correlations between a certain histological element and a feature could be seen, except for hMSH2 in yolk sac tumors. Although the limited number of cases investigated preclude any strong conclusions.

Our results add important findings towards a better understanding of treatment resistance in GCTs. We confirm a positive association between unfavourable treatment outcome and MMR deficiency and MSI, raising the question whether these factors could serve as both prognostic and predictive markers. This issue should be clarified by a translational approach in prospective clinical trials. In addition to identifying alterations that can potentially be used in screening and monitoring of treatment response, our analysis could provide a biological explanation for the response seen with the use of cytotoxic drugs with good activity in MSI tumors. Finally, the discovery of *BRAF* mutation V600E as the first gene mutation with a potential role in chemotherapy resistance in non-seminomatous GCTs holds promise for the future use and development of targeted therapies. Multi-kinase inhibitors targeting *BRAF*, like sorafenib, are clinically well established [15, 16], and anticancer agents, and substances that inhibit the downstream pathway involving the mitogen-activated protein kinase (MAPK) are in clinical development [17-19].

The results described above are accepted for publication in the Journal of Clinical Oncology [20].

References

1. Ulbright, T.M., *Germ cell neoplasms of the testis*. Am J Surg Pathol, 1993. **17**: p. 1075-1091.
2. Einhorn, L.H., *Curing metastatic testicular cancer*. Proc Natl Acad Sci U S A, 2002. **99**(7): p. 4592-5.
3. Mayer, F., et al., *Towards understanding the biological basis of the response to cisplatin-based chemotherapy in germ cell tumors*. Ann Oncol, 2003. **9**: p. 825-832.
4. Bartkova, J., et al., *DNA damage response in human testes and testicular germ cell tumours: biology and implications for therapy*. Int J Androl, 2007. **30**(4): p. 282-91; discussion 291.
5. Olasz, J., et al., *Influence of hMLH1 methylation, mismatch repair deficiency and microsatellite instability on chemoresistance of testicular germ-cell tumors*. Anticancer Res, 2005. **25**(6B): p. 4319-24.
6. Mayer, F., et al., *Microsatellite instability of germ cell tumors is associated with resistance to systemic treatment*. Cancer Res, 2002. **62**: p. 2758-2760.
7. Velasco, A., et al., *Mismatch repair expression in testicular cancer predicts recurrence and survival*. Int J Cancer, 2007.
8. Velasco, A., et al., *Microsatellite Instability and Loss of Heterozygosity Have Distinct Prognostic Value for Testicular Germ Cell Tumor Recurrence*. Cancer Biol Ther, 2004. **3**(11).
9. Deng, G., et al., *BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer*. Clin Cancer Res, 2004. **10**(1 Pt 1): p. 191-5.
10. Rajagopalan, H., et al., *Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status*. Nature, 2002. **418**(6901): p. 934.
11. Yuen, S.T., et al., *Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia*. Cancer Res, 2002. **62**(22): p. 6451-5.
12. Boland, C.R., et al., *A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer*. Cancer Res, 1998. **58**(22): p. 5248-57.
13. Garner, M.J., et al., *Epidemiology of testicular cancer: an overview*. Int J Cancer, 2005. **116**(3): p. 331-9.
14. Velasco, A., et al., *Mismatch Repair Gene Expression and Genetic Instability in Testicular Germ Cell Tumor*. Cancer Biol Ther, 2004. **3**(10).
15. Hiles, J.J. and J.M. Kolesar, *Role of sunitinib and sorafenib in the treatment of metastatic renal cell carcinoma*. Am J Health Syst Pharm, 2008. **65**(2): p. 123-31.
16. Strumberg, D. and S. Seeber, *Raf kinase inhibitors in oncology*. Onkologie, 2005. **28**(2): p. 101-7.
17. Allen, L.F., J. Sebolt-Leopold, and M.B. Meyer, *CI-1040 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK)*. Semin Oncol, 2003. **30**(5 Suppl 16): p. 105-16.
18. Rinehart, J., et al., *Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer*. J Clin Oncol, 2004. **22**(22): p. 4456-62.

19. Adjei, A.A., et al., *Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers*. J Clin Oncol, 2008. **26**(13): p. 2139-46.
20. Honecker, F., et al, *Microsatellite Instability, Mismatch Repair Deficiency, And BRAF Mutation In Treatment Resistant Germ Cell Tumors*. J Clin Oncol, accepted for publication 2008.