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Side-effects of GM-CSF Treatment in Advanced Testicular Cancer

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WITH THE increasing use of haematopoetic growth factors the benefits and the risks of these drugs need to be further determined. The biology and clinical applications of granulocyte (G) and granulocyte–macrophage (GM) colony-stimulating factor (CSF) have been described in many reviews [1, 2]. However, different frequencies of side-effects have been reported. In a recent phase I study, using subcutaneous application of bacterially synthesised GM-CSF in patients with different malignancies, the GM-CSF-related toxicity was regarded as minimal [3].

In a phase I/II study at Hannover University Medical School (Hannover, Germany) 59 patients with newly diagnosed metastatic advanced germ cell tumours were treated by dose-intensified chemotherapy with the platinum/etoposide/ifosfamide regimen (PEI). The 5-day regimen of PEI chemotherapy was repeated every 22 days for a total of four cycles, followed by subcutaneous GM-CSF (Shering Plough, Kenilworth, U.S.A.) starting the first day after chemotherapy (day 6) for 10 consecutive days. The first 38 patients received GM-CSF at a dose of 10 μg/kg bodyweight daily; the next 21 patients were treated with a lower dose of 5 μg/kg of GM-CSF. The incidence of side-effects of GM-CSF therapy is shown in Table 1. An interim analysis of the study results is given elsewhere [4].

Overall, 8 of 59 patients (13.5%) had to discontinue GM-CSF due to side-effects. In 1 patient with anaphylactic type reaction, who suffered from a prolonged phase of neutropenia after his fourth cycle of chemotherapy, GM-CSF was reinstituted with concomitant application of prednisolone (100 mg/day) which was well tolerated. Neither the frequency nor the severity of the anaphylactic type reactions in our patients was dependent upon the dose of GM-CSF used, either 10 or 5 μg/kg. In 3 patients with particularly severe reactions (two anaphylactic type, one cutaneous), who were further evaluated for plasma IgE levels and GM-CSF antibodies, no abnormalities were found. Biopsies from cutaneous reactions at the site of GM-CSF injection showed oedema of all skin layers and interstitial infiltration with lymphocytes and eosinophils, resembling the histological picture of skin eruptions in 3 patients with leukaemia receiving continuous systemic treatment with GM-CSF [5]. Overall, the incidence of skin reactions in patients with testicular cancer was low. In a placebo-controlled trial using GM-CSF for dose-intensive chemotherapy in patients with inflammatory breast cancer, all 7 patients randomised into the GM-CSF arm developed skin infiltrates at the site of injections [6].

The side-effects of GM-CSF treatment may not only be related to the underlying neoplastic disease but also to the type of cytostatic treatment used and its immunosuppressive potential. Further data on the side-effects of haematopoetic growth factors are clearly needed.

Table 1. GM-CSF-related side-effects in 59 testicular cancer patients treated with intensified PEI chemotherapy

Side-effects	Number of patients		
	5 μg/kg	10 μg/kg	Total
Anaphylactic type reaction (bronchospasm, myalgia, fever, skin reaction)	2	3	5 (8.4%)
Fever (without infection)	0	3	3 (5.1%)
Cutaneous reaction alone	1	1	2 (3.4%)

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Papillomavirus, p53 Alteration and Primary Carcinoma of the Vulva

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RECENT MOLECULAR studies on the role of human papilloma virus (HPV) in the genesis of genital carcinomas indicate as a

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Table 1.

Case no.	Diagnosis	HPV	p53 Mutations	p53 Immunostaining F	I	L
1	VIN III	16	Neg	0	1	N
2	VIN III	16	Neg	0	1	N
3	VIN III	16	Neg	0	0	0
4	VIN III	16	Neg	0	1	N
5*	VIN III	16	Neg	0	1	N
6†	ISCC	16	Neg	0	1	N
7	ISCC	16	Neg	3	3	N
8	ISCC	Neg	Neg	3	3	N+C
9	ISCC	Neg	Neg	3	3	N
10	ISCC	Neg	Neg	3	3	N
11	ISCC	Neg	codon 152	2	3	N
12	ISCC	Neg	codon 159	3	3	N
13	ISCC	Neg	exon 7‡	3	3	N
14	ISCC	Neg	codon 213	0	0	0

F: Stained cells frequency score: 0 ⩽ 10% of stained cells; 2 = 10–50%; 3 ⩾ 50%.
I: Stain intensity quantification: 0 = none; 1 = weak; 2 = moderate; 3 = strong.
L: location score: N = nuclear; C = cytoplasmic.
*Intraepithelial and †invasive components from the same case.
‡Determined only by SSCP.

crucial step the loss or alteration of host genes with negative growth regulatory function.

Current data point to p53 as a leading factor in the scenario. The loss of function of this cellular protein, known to be involved in the surveillance of DNA integrity and in this respect in the regulation of cell proliferation and differentiation, may be involved in the development of both HPV-positive and HPV-negative carcinomas.

In vitro p53 loss of function mediated by oncogenic HPV is gained through the formation of a molecular complex between viral E6 protein and wild-type p53 protein [1] whereas in HPV-negative carcinoma cell lines the same effect is obtained through the occurrence of somatic mutations [2]. Following these findings, HPV-positive carcinomas would express a wild-type p53, whereas HPV-negative carcinomas would display loss or alteration of p53.

To verify this concept *in vivo* we are characterising a series of vulval carcinomas for HPV typing and p53 analysis. As a model, carcinoma of the vulva shows two advantages in comparison to other sites recently investigated such as the anal skin [3] and the cervix [4]. Firstly, in the vulva a similar rate of HPV-positive and HPV-negative cases would allow a more reliable evaluation of the latter. Secondly, a definite prevalence of carcinomas with HPV 16 have been reported at this site, the oncovirus most extensively investigated to date *in vitro* [5, 6].

The preliminary results are as follows. DNA from 14 tissue samples of vulval intraepithelial neoplasia (VIN III) and invasive squamous cell carcinoma (ISCC) was investigated by Southern blotting with HPV type-specific probes for HPV types 6, 11, 16 and 18. The molecular study was accomplished by single strand conformation polymorphism (SSCP) analysis of PCR amplified p53 exons 5 to 9, followed, in 3 cases, by nucleotide sequence of the affected exon. Moreover, to detect p53 protein on paraffin sections we used a previously described modification of a conventional immunocytochemical technique [7] with two human p53-specific antibodies: polyclonal antibody (PAb) CM-1 and monoclonal antibody (MAb) DO-7.

The results are summarised in Table 1. The 5 cases of VIN III resulted positive for HPV 16 whereas the same result was obtained in only 2 (case numbers 6 and 7) of 9 cases of ISCC, one of which also had synchronous HPV-positive VIN III. SSCP analysis revealed no p53 alterations in all cases of VIN III (HPV-positive) and p53 mutations in 4 HPV-negative cases of ISCC.

The immunocytochemical analysis showed, regardless of the antibody used, a weak immunostaining in a few scattered nuclei (<10%) in all but one of the HPV-positive cases and, by contrast, a strong immunoreactivity in most of the nuclei in all but one of the HPV-negative cases (see Table 1).

The discrepancy between molecular and immunocytochemical analysis might be due to the limits of SSCP [8] as well as an increased expression of p53 protein without gene mutation [9]. In addition, the lack of p53 protein associated to a nonsense mutation in codon 213 has been already reported [10].

With the exception of one single HPV-positive case showing also an increased p53 expression, our preliminary results indicate an inverse relationship between p53 alterations and presence of HPV, and closely reflect the process of p53 degradation observed *in vitro* [1]. In addition, these results further strengthen the role of p53 in the pathogenesis of vulvar carcinoma which appears to proceed along at least two different pathways, depending upon the mode of p53 inactivation: indirectly by a HPV E6 interaction (mainly in VIN III) or directly by DNA mutation and/or altered expression (mostly in ISCC).

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