A Causal Role for the Human Tumor Antigen Preferentially Expressed Antigen of Melanoma in Cancer

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Abstract

Tumor antigens are of interest as diagnostic and prognostic markers and potential therapeutic targets. The tumor antigen preferentially expressed antigen of melanoma (PRAME) is frequently overexpressed in a wide variety of cancers and is a prognostic marker for clinical outcome. It has been shown recently that PRAME functions as a repressor of retinoic acid signaling. Here, we discuss this novel insight in the context of the increasing interest in tumor antigens as targets for therapy. (Cancer Res 2006; 66(22): 10639-42)

Expression of Preferentially Expressed Antigen of Melanoma in Cancer

Conceptually, tumor antigens can be divided into different groups based on their expression patterns and the presence of underlying gene mutations. They may be specific products of malignant cells due to mutations, translocations, and other distinct genetic events (e.g., the BCR/ABL fusion protein in leukemia). Second, tumor cells may express the wild-type version of proto-oncogenes at a higher level than their normal cellular counterparts (e.g., as a result of amplification as for *HER-2/neu* in breast cancer). Third, among the known tumor antigens are a class of nonmutated genes whose expression, with exception of testis and fetal tissues, seems to be mostly restricted to tumor cells. These cancer-testis antigens (CTA) include the MAGE, GAGE/PAGE, BAGE, LAGE/NY-ESO-1, and preferentially expressed antigen of melanoma (PRAME) families and they are mostly known for their association with melanoma.

The gene encoding PRAME was cloned by Ikeda et al. (1) as part of an investigation into tumor immune surveillance in a patient with a recurrent melanoma. This patient was found to express an antigen (PRAME), which elicited a cytotoxic T-cell-mediated immune response by autologous lymphocytes. The prevalence of *PRAME* expression is very high in melanomas: 88% of primary lesions and 95% of metastases (1). Gene expression profiling of the different stages of melanoma progression showed that *PRAME* is expressed in primary melanomas, but not in healthy skin tissue or in benign melanocytic lesions (nevi or moles), suggesting that PRAME expression may be an event in melanocyte transformation (2).

Besides melanoma, *PRAME* is frequently expressed in many different cancers and its expression correlates with prognosis and survival (Table 1). For instance, *PRAME* is expressed in non–small cell lung carcinomas, breast carcinomas, renal cell carcinomas,

head and neck cancers, Hodgkin's lymphomas, sarcomas, Wilms' tumors, and medulloblastomas (Table 1; refs. 1, 3–7). The expression of *PRAME* is also significant in the acute and chronic phases of both myelocytic and lymphocytic leukemias with reported frequencies of expression ranging from 17% to 42% in acute lymphoblastic leukemia (ALL) to 30% to 64% in acute myelogenous leukemia (AML; refs. 1, 8–12). A comparison of the gene signatures of the chronic, accelerated, and blast phases of chronic myelogenous leukemia (CML) has revealed that early in accelerated phase, before the accumulation of increased numbers of leukemia blast cells, new gene expression patterns occur, including an increase in *PRAME* expression (13).

Similar to melanoma, *PRAME* expression in neuroblastoma is extraordinarily common: reverse transcription-PCR (RT-PCR) screening detected *PRAME* expression in 93% of primary tumors and in 100% of patients with advanced disease (14). In neuroblastoma, highly significant associations exist between high *PRAME* expression and more advanced tumor stage, higher ages of patients at diagnosis, and poor clinical outcome of patients (14). Moreover, a large microarray-based study into the gene expression signatures of premenopausal breast cancers with good versus poor clinical outcomes identified *PRAME* expression as one of the molecular markers of poor prognosis. High *PRAME* expression correlated with an increased probability of development of metastases and with low disease-free and overall survival (3).

In contrast, a trend has been found that higher *PRAME* mRNA levels correlate with favorable prognosis and prolonged survival in childhood AMLs (11). Patients with the BCR/ABL t(9;22) (Ph+) and AML1/ETO t(8;21) translocations show particularly high PRAME mRNA levels (8, 15, 16). It cannot be excluded, however, that the correlation of PRAME expression with good prognosis is secondary to its correlation with these translocations (17). Indeed, it has been shown that *PRAME* is a BCR/ABL-inducible gene (15).

In contrast to other CTA, *PRAME* is also expressed in some normal tissues other than testis (Table 1). The high activation of *CTA* in testis may be explained by genome-wide DNA demethylation, which occurs in male germ-line cells. The cause of aberrant *PRAME* expression in malignancies is largely unknown. Because many studies have relied on mRNA-based techniques, it is clear that *PRAME* transcript levels are very highly induced in tumor tissues, but whether this is due to gene amplifications, enhanced transcription rates, or altered mRNA turnover has not been addressed. Genetic alterations, such as mutations or translocations in *PRAME*, have not been reported.

Surprisingly, a large family of *PRAME*-like genes and pseudogenes have evolved in the human genome (18). A comprehensive prediction of human *PRAME* homologues yielded a total of 22 *PRAME* genes and 10 pseudogenes, which have evolved recently in evolution through extensive gene duplications (18). However, very little is known about their expression and their possible functions. The mouse genome contains multiple *PRAME*-like genes in an

Table 1. Expression of PRAME in cancers and in normal tissues

Tissue type	Percentage	References
Melanoma		
Primary lesion	88	(1)
Metastasis	95	(1)
Mammary carcinoma	27	(1, 3)
Lung		
Non–small cell lung carcinoma		
Adenocarcinoma	46	(1)
Squamous cell carcinoma	78	(1)
Leukemia		
ALL	17-42	(1, 8, 9, 12)
AML	30-64	(1, 8–11)
CML	n.d.	(13)
Progressive acute megakaryoblastic		
leukemia of Down's syndrome	n.d.	(26)
Hodgkin's lymphoma	n.d.	(5)
Medulloblastoma	30-74	(7)
Neuroblastoma		
Primary lesion	93	(14)
Advanced disease	100	(14)
Head and neck tumor	39	(1)
Renal carcinoma	41	(1, 4)
Wilms' tumor	n.d.	(6)
Sarcoma	39	(1)
Multiple myeloma	n.d.	(27)
Normal tissue		
Testis		(1)
Endometrium		(1)
Bone marrow/CD34^{+} progenitors		(11)
Ovary		(1)
Adrenals		(1)

NOTE: There is anecdotal evidence for expression of PRAME in additional normal tissues and tumor types in the National Center for Biotechnology Information database of serial analysis of gene expression analysis for PRAME (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.30743).

Abbreviation: n.d., not determined.

orthologous region, but it is unclear if a functional mouse orthologue of human *PRAME* exists.

PRAME Function

The data described above raise the question whether activation of *PRAME* is causally implicated in oncogenic transformation or whether it is a mere reporter of progressive disease. Recent data indicate that PRAME may be instrumental in disease progression by interfering with retinoic acid (RA) receptor (RAR) signaling (19). RA signaling is essential in development, cell fate determination, and tissue homeostasis. RA induces transcription of a set of target genes by binding to and activation of its receptor, resulting in differentiation and cell cycle arrest in responsive cells (20). Loss of RA responsiveness is therefore beneficial to cancer cells. The amino acid sequence of PRAME reveals the presence of seven putative nuclear receptor boxes in the protein. Nuclear receptor boxes (LXXLL motifs, where L is leucine and X is any amino acid) are often present in proteins that bind to and modulate the activities of nuclear hormone receptors. When tested for its ability to repress transactivation by several class I and class II nuclear hormone receptors, it was found that PRAME only acts to repress RAR signaling (19). However, these data do not exclude that PRAME has additional functions or that it plays roles in other nuclear receptor signaling pathways.

RAR represses target gene transcription in the absence of its ligand and activates transcription after binding of RA. PRAME was found to bind directly to RAR and inhibit RAR-mediated trans-activation, even in the presence of RA, thus acting as a dominant inhibitor of the RAR pathway (19). Ectopic expression of PRAME in RA-sensitive cells was shown to confer resistance to RA-induced growth arrest, differentiation, and apoptosis. Moreover, knockdown of PRAME by RNA interference (RNAi) in melanoma cells, which are invariably RA resistant, restored sensitivity to RA and induction of the target genes *RARβ* en *p21*. Importantly, melanoma xenografts with PRAME knockdown could be treated with RA resulting in significantly smaller tumor sizes compared with tumors without PRAME knockdown. Together, these results established a dominant inhibitory effect of PRAME on RAR signaling (Fig. 1; ref. 19).

Suppression of the proliferation arrest and differentiation normally induced by RA may explain why *PRAME* expression is positively selected for during oncogenesis. RARα is involved in the promyelocytic leukemia (PML)-RARα and the more rare PLZF-RARα translocations that cause pediatric APL. The resulting fusion proteins encode functionally altered receptors, which are constitutive repressors of RA-induced differentiation of leukemic blasts (20). The overexpression of *PRAME* in solid tumors may have a similar effect on cell physiology and thereby phenocopy the RARα translocations in APL. It remains to be elucidated whether tumor cells are "addicted" to the presence of high PRAME levels. Based on the current limited knowledge on PRAME function, melanoma represents arguably the strongest case for PRAME being causally implicated in the malignant phenotype. The extremely high prevalence of *PRAME* expression in melanoma (1) and the effects of knockdown of PRAME by RNAi in melanoma (19) support this idea.

Given the differential correlation between *PRAME* expression and prognosis in solid tumors versus childhood acute leukemias, the role of PRAME in leukemias may be different from its function in solid tumors. It has been shown that transient overexpression of PRAME can induce a caspase-independent cell death (21). Stable expression resulted in a decreased proliferation rate. Furthermore, knockdown of PRAME by RNAi increased the tumorigenicity of K562 leukemic cells in nude mice (21). These data raise the possibility that PRAME has a different role in oncogenic transformation of solid tumors compared with hematologic malignancies.

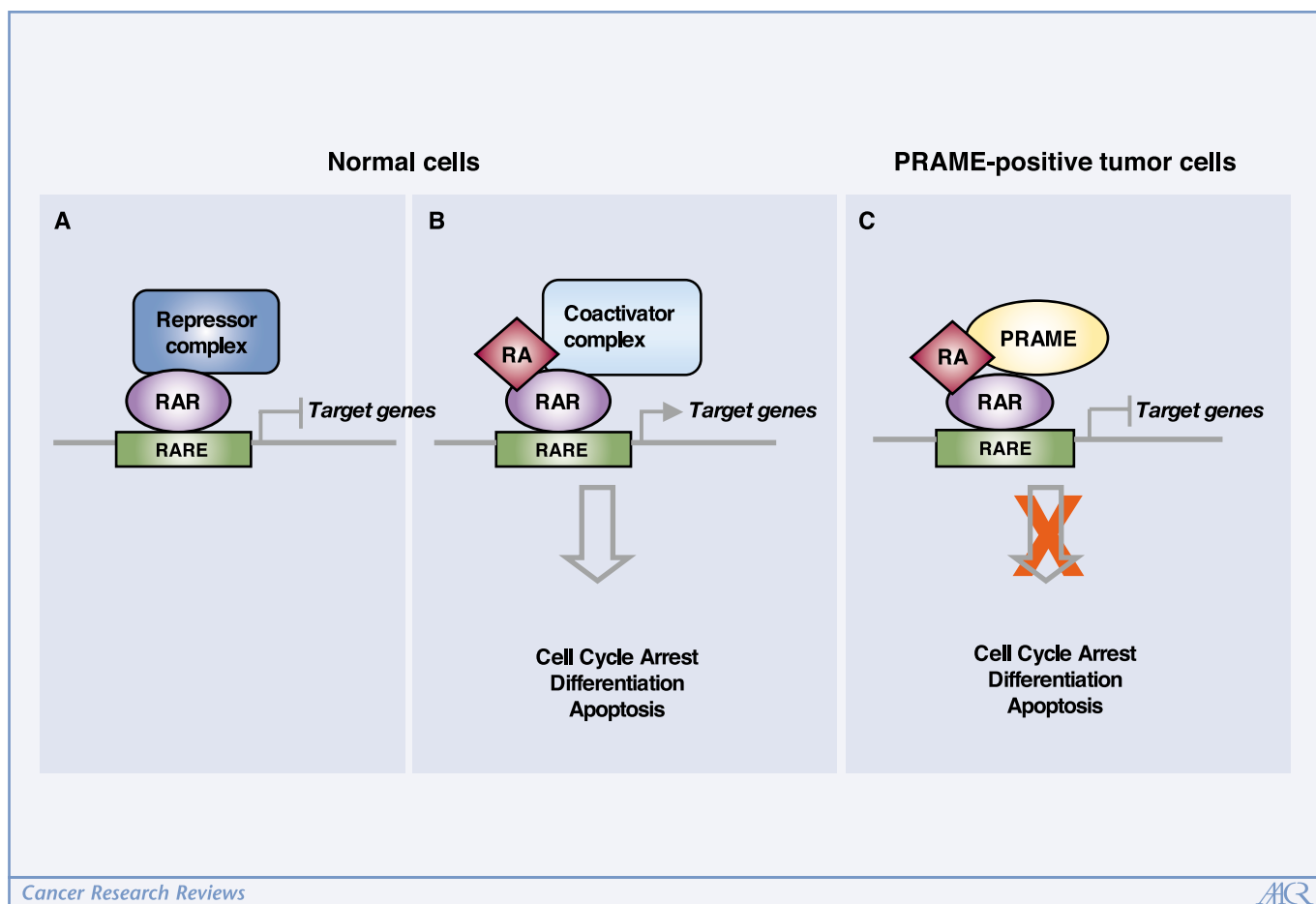## Implications for Cancer Treatment

Tumor antigens can be exploited in two different ways: they can be used to detect the presence of residual cancer cells and they can serve as targets for therapy. Detection of minimal residual disease (MRD) is critical in, for example, acute leukemias as the persistence of leukemic blast cells represents a serious risk of relapse. Various methods are used for the detection of MRD and several genes have been tested for their suitability as markers of MRD by qualitative and quantitative RT-PCR techniques. *PRAME* is one of

the molecular markers that are being tested in AML and seems to be a promising diagnostic marker for the detection of MRD in AML (22).

For the development of more effective and less toxic cancer therapies, increased selectivity of therapeutic agents is urgently needed. Tumor-specific proteins or pathways that the tumor cells depend on are preferred as therapeutic targets. Some notable successes are drugs targeting the *BCR/ABL* oncogene in CML and the *HER-2/neu* oncogene in breast cancer. The tumor antigen PRAME has two properties in common with these highly successful drug targets. First, high *PRAME* expression is mostly restricted to tumor cells. Second, its expression contributes to the oncogenic phenotype, as PRAME confers a selective advantage to tumor cells by virtue of its inhibitory effect on RA signaling. Such a causal role for a tumor antigen in oncogenesis adds to its potential as a target for therapy, as escape from therapy through down-regulation of antigen expression would be disadvantageous for the tumor cell. However, the normal tissues, in which *PRAME* is expressed, may pose problems for systemic therapeutic targeting of PRAME-positive cancer cells, especially because PRAME mRNA has been detected in CD34^{+} progenitor cells and bone marrow (Table 1; ref. 11). Preclinical *in vivo*

experiments to determine the toxicity profile of PRAME-directed therapies are complicated by the lack of a clear rodent orthologue of PRAME (18).

Two strategies could be considered to use PRAME as a specific target in cancer. It is conceivable that, despite the intrinsic immunogenicity of PRAME, immune responses to solid tumors are often abrogated by immunosuppressive mechanisms/factors produced by the tumor cells. Immunotherapies could be designed to boost the T-cell-mediated immune response to PRAME-positive tumor cells. Immune therapy may spare normal cells, as PRAME-positive testes cells are not good targets for T cells due to the lack of direct contact with the immune cells and the lack of HLA class I expression on the surface of germinal cells. Furthermore, the low expression of *PRAME* in other normal tissues may not be sufficient for CTL recognition. PRAME-specific lysis of multiple tumor cell lines by CTL in an HLA-restricted manner has been shown (23). However, whether patients can be stimulated to consistently mount a robust immunologic response to this antigen has yet to be established. It has been shown recently that the expression of *PRAME* can induce antileukemic immune responses in AML. In the majority of AML patients in complete remission, specific T-cell responses to PRAME peptides were detected, in contrast to

Figure 1. Model of PRAME function in cancer. *A*, in normal cells, in the absence of ligand, RARα recruits repressor complexes to the promoters of its target genes to repress gene transcription. *B*, on binding of RA, the repressor complexes are released and coactivators are recruited to induce gene transcription, resulting in differentiation, proliferation arrest, and/or apoptosis. *C*, in PRAME-positive tumor cells, PRAME interacts with RARα in the presence of ligand and blocks gene transcription. Constitutive repression of RA signaling by PRAME may be one of the genetic events in oncogenesis and represent a new mechanism of RA resistance in human cancers. *RARE,* RA-responsive element.

AML patients with refractory disease (17). This suggests that T-cell responses can contribute to tumor eradication, even in the absence of additional stimulation of the immune system. Heterogeneous intratumor expression of CTA may hamper the effectiveness of CTA-directed vaccination and RT-PCR has revealed a highly heterogeneous expression of MAGE, GAGE, and PRAME among human melanoma clones derived from the same lesion (24). Immunotherapeutic approaches for AML have been developed, which use dendritic cells generated from PRAME-positive AML blasts (AML-dendritic cells). These AML-dendritic cells up-regulated costimulatory molecules and were recognized by specific T cells. The first clinical study targeting PRAME used autologous AML-dendritic cells, which were injected into AML patients. This resulted in increased T-cell numbers specifically recognizing a PRAME-derived peptide (25).

Alternatively, small molecule-based strategies to target PRAME in cancer can be envisioned. However, PRAME has not been shown to possess intrinsic enzymatic activity, which could be targeted by small-molecule drugs. In addition, the small hydrophobic α-helical interaction surface between PRAME and RAR is a difficult target for drug development. One possible starting point could be the finding that PRAME requires the histone methyltransferase activity of the polycomb protein EZH2 to mediate transcriptional repression (19). Therefore, small-molecule inhibitors of the histone methyltransferase activity of EZH2 may have especially potent effects in PRAME-positive tumor cells.

Several important issues remain to be solved for PRAME. First, which signaling pathways are targeted in the various types of cancer, in which *PRAME* is expressed? PRAME has been shown to inhibit RA signaling in melanoma (19), and it will be of interest to know if PRAME blocks RA signaling in other cancers in which it is expressed. Alternatively, the protein may be able to interfere with other pathways through one or more of its remaining nuclear receptor boxes because PRAME has seven such motifs and only one of these is used to interact with RAR (19). Second, it remains unclear how *PRAME* expression is reactivated during oncogenesis. In general, CTA expression can be induced with 5-aza-deoxycytidine (a DNA-hypomethylating agent; ref. 24), suggesting that epigenetic control mechanisms underlie the silencing of *PRAME* in most adult tissues. PRAME was up-regulated in *BCR/ABL*-transduced cells, suggesting a role for BCR/ABL in the activation of PRAME (15). Many more of these oncogene or pathway-induced mechanisms of PRAME up-regulation may exist and it will be important to identify the mechanisms that cause *PRAME* to become selectively activated during oncogenesis.

Acknowledgments

References

1. Ikeda H, Lethe B, Lehmann F, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity 1997;6:199–208.
2. Haqq C, Nosrati M, Sudilovsky D, et al. The gene expression signatures of melanoma progression. Proc Natl Acad Sci U S A 2005;102:6092–7.
3. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
4. Neumann E, Engelsberg A, Decker J, et al. Heterogeneous expression of the tumor-associated antigens RAGE-1, PRAME, and glycoprotein 75 in human renal cell carcinoma: candidates for T-cell-based immunotherapies? Cancer Res 1998;58:4090–5.
5. Willenbrock K, Kuppers R, Renne C, et al. Common features and differences in the transcriptome of large cell anaplastic lymphoma and classical Hodgkin's lymphoma. Haematologica 2006;91:596–604.
6. Li CM, Guo M, Borczuk A, et al. Gene expression in Wilms' tumor mimics the earliest committed stage in the metanephric mesenchymal-epithelial transition. Am J Pathol 2002;160:2181–90.
7. Boon K, Edwards JB, Siu IM, et al. Comparison of medulloblastoma and normal neural transcriptomes identifies a restricted set of activated genes. Oncogene 2003;22:7687–94.
8. van Baren N, Chambost H, Ferrant A, et al. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. Br J Haematol 1998;102:1376–9.
9. Paydas S, Tanriverdi K, Yavuz S, Disel U, Baslamisli F, Burgut R. PRAME mRNA levels in cases with acute leukemia: clinical importance and future prospects. Am J Hematol 2005;79:257–61.
10. Greiner J, Ringhoffer M, Taniguchi M, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. Int J Cancer 2004;108:704–11.
11. Steinbach D, Hermann J, Viehmann S, Zintl F, Gruhn B. Clinical implications of PRAME gene expression in childhood acute myeloid leukemia. Cancer Genet Cytogenet 2002;133:118–23.
12. Steinbach D, Viehmann S, Zintl F, Gruhn B. PRAME gene expression in childhood acute lymphoblastic leukemia. Cancer Genet Cytogenet 2002;138:89–91.
13. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A 2006;103:2794–9.
14. Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high-stage neuroblastoma and associated with poor outcome. Clin Cancer Res 2004;10:4307–13.
15. Watari K, Tojo A, Nagamura-Inoue T, et al. Identification of a melanoma antigen, PRAME, as a BCR/ABL-inducible gene. FEBS Lett 2000;466:367–71.
16. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. Blood 2004;104:3679–87.
17. Greiner J, Schmitt M, Li L, et al. Expression of tumor-associated antigens in acute myeloid leukemia: implications for specific immunotherapeutic approaches. Blood. Epub 2006 Aug 24.
18. Birtle Z, Goodstadt L, Ponting C. Duplication and positive selection among hominin-specific PRAME genes. BMC Genomics 2005;6:120.
19. Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell 2005;122:835–47.
20. Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. Nat Rev Cancer 2001;1:181–93.
21. Tajeddine N, Gala JL, Louis M, Van Schoor M, Tombal B, Gailly P. Tumor-associated antigen preferentially expressed antigen of melanoma (PRAME) induces caspase-independent cell death in vitro and reduces tumorigenicity in vivo. Cancer Res 2005;65:7348–55.
22. Steinbach D, Schramm A, Eggert A, et al. Identification of a set of seven genes for the monitoring of minimal residual disease in pediatric acute myeloid leukemia. Clin Cancer Res 2006;12:2434–41.
23. Griffioen M, Kessler JH, Borghi M, et al. Detection and functional analysis of CD8⁺ T cells specific for PRAME: a target for T-cell therapy. Clin Cancer Res 2006;12:3130–6.
24. Sigalotti L, Fratta E, Coral S, et al. Intratumor heterogeneity of cancer/testis antigens expression in human cutaneous melanoma is methylation-regulated and functionally reverted by 5-aza-2'-deoxycytidine. Cancer Res 2004;64:9167–71.
25. Li L, Giannopoulos K, Reinhardt P, et al. Immunotherapy for patients with acute myeloid leukemia using autologous dendritic cells generated from leukemic blasts. Int J Oncol 2006;28:855–61.
26. McElwaine S, Mulligan C, Groet J, et al. Microarray transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukaemia (M7) in Down's syndrome, revealing PRAME as a specific discriminating marker. Br J Haematol 2004;125:729–42.
27. Pellat-Deceunynck C, Mellerin MP, Labarriere N, et al. The cancer germ-line genes MAGE-1, MAGE-3 and PRAME are commonly expressed by human myeloma cells. Eur J Immunol 2000;30:803–9.