thalassemia major: a randomized clinical trial. *Blood Cells, Molecules and Diseases*, **28,** 196–208.

Miskin, H., Yaniv, I., Berant, M., Hershko, C. & Tamary, H. (2003) Reversal of cardiac complications in thalassemia major by long-term intermittent daily intensive iron chelation. *European Journal of Haematology*, **70,** 398–403.

Piga, A., Gaglioti, C., Fogliacco, E. & Tricta, F. (2003) Comparative effects of deferiprone and deferoxamine on survival and cardiac disease in patients with thalassemia major: a retrospective analysis. *Haematologica*, **88,** 489–496.

Keywords: heart disease, thalassaemia, deferiprone, deferoxamine.

doi:10.1111/j.1365-2141.2004.05196.x

Aberrant somatic hypermutation in post-transplant lymphoproliferative disorders

Monoclonal post-transplant lymphoproliferative disorders (PTLD) include polymorphic PTLD (P-PTLD), diffuse large B-cell lymphoma (DLBCL) and Burkitt/Burkitt-like lymphoma (BL/BLL). Most PTLD originate from germinal centre (GC)-experienced B cells that have undergone the physiological somatic hypermutation (SHM) process targeting immunoglobulin variable (IgV) genes (Capello *et al*, 2003; Timms *et al*, 2003).

Aberrant SHM is a pathogenetic mechanism implicated in DLBCL of immunocompetent hosts and acquired immunodeficiency syndrome (AIDS)-related lymphomas that targets the 5′ region, including coding sequences, of multiple proto-oncogenes relevant to lymphomagenesis, namely *PIM-1*, *PAX-5*, *RhoH/TTF* and *c-MYC* (Pasqualucci *et al*, 2001). Mutations are somatic in origin, occur independent of chromosomal translocation, and share features of IgV SHM. In contrast to IgV SHM, aberrant SHM does not occur at a significant level in normal GC B-cells, suggesting that aberrant SHM in lymphoma results from a tumour-specific malfunction of the SHM machinery (Pasqualucci *et al*, 2001).

The knowledge that PTLD derive from B cells that have experienced the physiological SHM process prompted our analysis of aberrant SHM in 25 monoclonal PTLD (five P-PTLD; 18 DLBCL; two BL/BLL) consecutively collected at diagnosis from solid organ transplant recipients (Table I). Direct DNA sequencing analysis of *PIM-1*, *PAX-5*, *RhoH/TTF* and *c-MYC* was performed as previously reported (Pasqualucci *et al*, 2001). The study was approved by the institutional review board and, where appropriate, informed consent was obtained.

Mutations in at least one of the four proto-oncogenes were found in seven of 25 (28·0%) PTLD, all represented by DLBCL (Table I). When considering solely DLBCL, aberrant hypermutation occurred in seven of 18 cases (38·8%). Mutations in more than one gene were restricted to one single case (case 3467), carrying mutations in *PAX-5* and *c-MYC* (Table I). *PAX-5* was mutated in four of 25 cases (16·0%), *RhoH*/TTF in one of 25 cases (4·0%), and *c-MYC* in three of 25 cases (12·0%). The observed changes may be ascribed to the lymphoma clone, rather than to contaminating B cells because the proportion of tumour cells in all PTLD was ≥ 70% and our experimental strategy enabled detection only of mutations that were present in >10% of cells. Aberrant SHM occurred in three of 13 Epstein–Barr virus (EBV)-positive and four of 12 EBV-negative PTLD, suggesting independence from tumour viral infection. Based on nonparametric statistical analysis (Kruskal–Wallis test and Mann–Whitney test with Bonferroni adjustment for multiple comparison), the frequency of aberrant SHM in PTLD/DLBCL did not differ from that of AIDS-DLBCL but was lower than that reported in DLBCL of immunocompetent hosts ($P < 0{\cdot}05$) (this study and our unpublished observations).

A total of 30 mutations were found in seven PTLD (Table I). Most mutations included single basepair substitutions ($n = 29$; 16 transitions and 13 transversions), whereas only one deletion of a short DNA stretch was observed (Table I). The observed transition/transversion ratio of 1·23 (expected 0·5), a general feature of physiological SHM, reflected that of DLBCL of immunocompetent hosts and of AIDS-non-Hodgkin's lymphoma (Pasqualucci *et al*, 2001; Gaidano *et al*, 2003). The *c-MYC* mutation of case 3522 (ATC→GTC) led to an Ile129Val amino acid substitution within the gene transactivation domain, a mutational hotspot in translocated c-*MYC* alleles in BL and mouse plasmacytoma (Dang, 1999).

The association of PTLD with aberrant SHM expands the types of aggressive lymphomas marked by this molecular abnormality. Missense mutations in the *c-MYC* transactivation domain may deregulate its function by interfering with multiple *c-MYC* properties (Dang, 1999), whereas mutations in *PAX-5*, *RhoH/TTF* and *c-MYC* 5′ regulatory regions may influence the expression of these genes in a manner similar to that reported for *BCL-6* in B-cell lymphoma (Pasqualucci *et al*, 2003). Given the relevant role of *PAX-5* in B-cell differentiation, of *RhoH/TTF* in signal transduction, and of *c-MYC* in

Table I. Clinical and molecular features of PTLD.

Case	Histology*	Sex†	TX†	Age at TX (years)	Immune suppression†	Interval from TX (months)	Tumour site	Stage at diagnosis	Outcome, months from PTLD	EBV	IgV_H SHM (% mutations)	Aberrant SHM			
												PAX-5‡	*RhoH/TTF*‡	*PIM-1*‡	*c-MYC*‡
3464	P-PTLD	M	Liver	59	C + A	5	Liver	I	Alive at 27 mo†	+	0	–	–	–	–
3515	P-PTLD	M	Heart	13	C + A	79	Lymph node	II	Death at 18 mo	–	10·3	–	–	–	–
3516	P-PTLD	M	Heart	18	C + A	22	Lymph node	II	Death at 20 mo	+	NA	–	–	–	–
3519	P-PTLD	M	Heart	59	C + A	84	Lymph node	III	Death at 6 mo	–	10·3	–	–	–	–
3521	P-PTLD	F	Heart	14	C + A	51	Lymph node	I	Alive at 41 mo	+	1·0	–	–	–	–
2898	DLBCL, C	M	Heart	34	C + A	81	Lymph node	III	Alive at 52 mo	–	11·8	–	–	–	–
3467	DLBCL, C	M	Heart	52	C + A	128	Lymph node	IV	Death at 1 d	+	1·80	A1305G	–	–	G3013A
3518	DLBCL, C	M	Heart	55	C + A	60	Lymph node	II	Death at 8 mo	+	4·41	–	–	–	T2671G, G2709C, A2884G, A2910G, A2922C, T2928G, A2930T, A2931C, G2933C, G2936A, C2973G, G3004A, T3005C, A3010C, G3032T, T3067C, T3069G, A3099G, G3103T, A3143G, C3226G, T3338C
3522	DLBCL, C	M	Liver	1	FK506	29	Jejunum	II	Alive at 41 mo	–	6·87	–	–	–	A4905G
3523	DLBCL, C	F	Heart	12	C + A	102	Lymph node	I	Alive at 29 mo	–	2·71	–	–	–	–
3524	DLBCL, C	M	Heart	21	C + A	78	Skin	IV	Death at 12 mo	–	14·9	–	–	–	–
3527	DLBCL, C	M	Kidney	28	C + A	51	Lymph node	III	Death at 3 mo	–	7·12	–	–	–	–
3528	DLBCL, C	M	Heart	16	C + A	95	Lymph node	II	Death at 4 mo	–	8·11	C953T	–	–	–
3530	DLBCL, C	M	Heart	58	C + A	144	Lymph node	II	Alive at 15 mo	–	24·1	–	A675G, A730G	–	–
2892	DLBCL, I	M	Heart	44	FK506 + A	42	Retroperitoneum	I	Alive at 137 mo	+	8·59	–	–	–	–
2895	DLBCL, I	M	Kidney	46	C + A	72	Lymph node	III	Autoptic diagnosis	+	7·20	–	–	–	–
2913B	DLBCL, I	M	Heart	38	C + A	158	Tonsil	I	Death at 10 mo	+	NA	–	–	–	–
3459	DLBCL, I	M	Lung	27	C + A	128	Lymph node	IV	Alive at 25 mo	+	17·6	–	–	–	–
3476	DLBCL, I	M	Heart	53	C + A	108	Skin	I	Death at 38 mo	+	8·92	–	–	–	–
3517	DLBCL, I	M	Heart	49	C + A	84	Lymph node	IV	Autoptic diagnosis	–	7·80	Δ1241-1248	–	–	–
3520	DLBCL, I	M	Kidney	15	C + P	156	Kidney	I	Death at 4 mo	+	10·7	–	–	–	–
3525	DLBCL, I	F	Kidney	21	C + A	108	Spleen	IV	Death at 1 mo	–	2·12	–	–	–	–
3529	DLBCL, I	F	Heart	6	C + A	72	Lymph node	III	Alive at 21 mo	+	NA	C1018T	–	–	–
2890	BL/BLL	M	Heart	27	C + A	106	Tonsil	I	Death at 22 mo	–	12·0	–	–	–	–
3526	BL/BLL	M	Heart	5	C + A	94	Jaw	I	Death at 18 mo	+	1·70	–	–	–	–

*P-PTLD, polymorphic PTLD; DLBCL, C, diffuse large B-cell lymphoma, centroblastic; DLBCL, I, diffuse large B cell lymphoma, immunoblastic; BL/BLL, Burkitt lymphoma/Burkitt-like lymphoma.
†M, male; F, female; TX, transplantation; mo, months; C, cyclosporine A; A, azathioprine; P, prednisone.
‡, – germline sequence. Where present, mutations are indicated by nucleotide substitution and location.

B-cell growth and fate, further studies are required to formally clarify the pathogenetic contribution of aberrant hypermutation of these genes in PTLD development.

Acknowledgements

The study was supported by Cofin 2002, MIUR, Rome, Italy; Progetto Strategico Oncologia, CNR-MIUR, Rome, Italy; Ricerca Finalizzata 2002, Ministero della Salute, Rome, Italy; Regione Piemonte, Torino, Italy; and Novara-AIL Onlus, Novara, Italy. EB is supported by a fellowship from Fondazione 'Piera Pietro e Giovanni Ferrero, Alba, Italy. LP is a Special Fellow of the Leukemia and Lymphoma Society.

Michaela Cerri[1]
Daniela Capello[1]
Giuliana Muti[2]
Alessandro Rambaldi[3]
Marco Paulli[4]
Annunziata Gloghini[5]
Eva Berra[1]
Clara Deambrogi[1]
Davide Rossi[1]
Silvia Franceschetti[1]
Annarita Conconi[1]
Enrica Morra[2]
Laura Pasqualucci[6]
Antonino Carbone[5]
Gianluca Gaidano[1]

[1]Haematology Unit, Department of Medical Sciences and IRCAD, Amedeo Avogadro University of Eastern Piedmont, Novara, [2]Division of Haematology, Ospedale Niguarda Ca' Granda, Milan, [3]Division of Haematology, Ospedali Riuniti, Bergamo, [4]Department of Pathology, IRCCS Policlinico San Matteo/University of Pavia, Pavia, [5]Division of Pathology, Centro di Riferimento Oncologico, Istituto Nazionale Tumori, IRCCS, Aviano, Italy; and [6]Institute for Cancer Genetics, Columbia University, New York, NY, USA.
E-mail: gaidano@med.unipmn.it

References

Capello, D., Cerri, M., Muti, G., Berra, E., Oreste, P., Deambrogi, C., Rossi, D., Dotti, G., Conconi, A., Viganò, M., Magrini, U., Ippoliti, G., Morra, E., Gloghini, A., Rambaldi, A., Paulli, M., Carbone, A. & Gaidano, G. (2003) Molecular histogenesis of posttransplantation lymphoproliferative disorders. *Blood*, **102,** 3775–3785.

Dang, C.V. (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Molecular and Cellular Biology*, **19,** 1–11.

Gaidano, G., Pasqualucci, L., Capello, D., Berra, E., Deambrogi, C., Rossi, D., Larocca, L.M., Gloghini, A., Carbone, A. & Dalla-Favera, R. (2003) Aberrant somatic hypermutation in multiple subtypes of AIDS-associated non-Hodgkin lymphoma. *Blood*, **102,** 1833–1841.

Pasqualucci, L., Neumeister, P., Goossens, T., Nanjangud, G., Chaganti, R.S., Kuppers, R. & Dalla-Favera, R. (2001) Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature*, **412,** 341–346.

Pasqualucci, L., Migliazza, A., Basso, K., Houldsworth, J., Chaganti, R.S. & Dalla-Favera, R. (2003) Mutations of the BCL-6 proto-oncogene disrupt its negative autoregulation in diffuse large B-cell lymphoma. *Blood*, **101,** 2914–2923.

Timms, J.M., Bell, A., Flavell, J.R., Murray, P.G., Rickinson, A.B., Traverse-Glehen, A., Berger, F. & Delecluse, H.J. (2003) Target cells of Epstein-Barr-virus (EBV)-positive post-transplant lymphoproliferative disease: similarities to EBV-positive Hodgkin's lymphoma. *Lancet*, **361,** 217–223.

Keywords: malignant lymphomas, molecular pathogenesis, immunodeficiency, somatic mutation, proto-oncogenes.

doi:10.1111/j.1365-2141.2004.05203.x

PK Aarau: first homozygous nonsense mutation causing pyruvate kinase deficiency

Pyruvate kinase (PK) deficiency of erythrocytes (OMIM 266200) is the most common cause of hereditary non-spherocytic haemolytic anaemia due to defective glycolysis (Hirono *et al*, 2001). PK is encoded by two genes, *PKLR* and *PKM*, expressing four isozymes in mammalian tissues: L-type (hepatic) and R-type (erythrocytic) encoded by *PKLR* and M1- (muscle and brain) and M2- (fetal and most adult tissues) types encoded by *PKM*. The disorder is transmitted as an autosomal recessive trait.

So far, at least 133 different mutations have been identified in PK deficiency (Bianchi & Zanella, 2000). During the last few years several homozygous PK null mutations have been identified, one large deletion, two one base deletions and one transition at a splice site (Zanella & Bianchi, 2000). To date, no