Clonal Immunoglobulin Gene Rearrangements and Normal T-Cell Receptor, bcl-2, and c-myc Genes in Primary Cutaneous B-Cell Lymphomas

Domenico Delia, Maria G. Borrello, Emilio Berti, Marco A. Pierotti, Daniela Biassoni, Raffaele Gianotti, Elvio Alessi, Maria G. Rizzetti, Ruggero Caputo, and Giuseppe Della Porta

Division of Experimental Oncology A, Istituto Nazionale Tumori, Via G. Venezia 1, 20133 Milano, Italy.

ABSTRACT

Fourteen cases of primary cutaneous B-cell lymphomas were investigated at the immunohistochemical and molecular level to further characterize this newly defined entity. Neoplastic cells from all cases, phenotyped with a panel of monoclonal antibodies, were positive for HLA-DR, for the B-cell markers CD19, CD22, but not CD23 (except one case), and negative for the T-cell marker CD2. Monoclonal immunoglobulin light chains were demonstrated in six cases. The reactivity with the Ki-67 monoclonal antibody indicated that the neoplastic cells are proliferating. In five biopsies the presence of dendritic cells infiltrating the neoplastic areas was revealed using the monoclonal antibody Kim4b.

By Southern blot analysis, clonal rearrangement of the immunoglobulin heavy chain gene (involving one or both alleles) was shown in 12 of 14 cases and of the light chain genes in 13 cases. The bcl-2 oncogene, normally involved in nodal follicular lymphomas, was in germ-line configuration. The monoclonal and the ß and γ chain genes of the T-cell receptor were also in the germ-line configuration. None of the cases presented Epstein-Barr virus sequences.

These data indicate that primary cutaneous lymphomas of B-cell origin share morphological and phenotypic similarities with the nodal B-cell lymphomas of follicular histotype, are proliferating, and express in 45% of cases clear monoclonal immunoglobulin light chains; the molecular analysis confirms the B-cell derivation and the monoclonal nature of this neoplasia; it also shows that neither bcl-2 nor c-myc oncogenes are involved and that no inappropriate rearrangements of the T-cell receptor genes are found in this lymphoma.

INTRODUCTION

Primary cutaneous B-cell lymphoma of germinal centre cell origin is a rare, poorly characterized disease presenting localized skin nodules and plaques made up of a dense non-epidermotropic follicular and/or diffuse lymphoid infiltrate around vessels and adnexa (1). The disease is prevalent among adult patients, and has generally a very slow progressive course with systemic involvement even after prolonged follow-up (1, 2). Although for many years this entity has been considered as being either a true histiocytic or T-cell lymphoma, (3, 4), only recently, with the use of monoclonal antibodies and immunohistochemical techniques, has B-cell origin of the proliferating cells been demonstrated (5–7); moreover monoclonal immunoglobulin light chains, detected in some cases, suggest clonal origin of the disease.

Despite these findings, little is known so far about the pathogenetic mechanisms of this disease, contrary to nodal lymphomas where extensive investigations have shown that several cytogenetic and molecular abnormalities are consistently associated with morphologically and histologically different B- and T-cell neoplasms. These include, among the B-cell neoplasms, the t(8;14) translocation of Burkitt’s lymphomas, the t(11;14) translocation of B-CLL, and the t(14;18) of follicular lymphomas (8, 9). The analysis of the breakpoints of the chromosomal rearrangements has served to identify genes important in inducing the tumor or affecting its progression (10, 11). Furthermore, the development of probes specific for such breakpoints can overcome the difficulties that can sometimes hamper the cytogenetic studies.

Taking advantage of these techniques, we decided to investigate the molecular aspect of primary cutaneous lymphomas of B-cell phenotype to confirm the B-cell lineage and clonality by structural analysis of the immunoglobulin genes. In addition, it was of interest to determine whether the cases presented abnormalities of protooncogenes involved in other B-NHL, such as c-myc, implicated in Burkitt’s lymphomas (12) and bcl-2, a breakpoint region occurring in follicular B-NHL (13), as well as the ß chain of the TCR complex, whose “inappropriate” rearrangement has been detected in some B-cell lymphomas (14).

MATERIALS AND METHODS

Patients. Fourteen patients were included in this study, six females and eight males, median age 57 (range, 26–28), seven of them presenting skin nodules or plaques localized on the back, three on the head, two on the thorax, and two on the legs. Clinical examination revealed involvement of superficial satellite lymph node in two cases while no abnormalities were found in the remaining. The chest X-ray, computerized axial tomography of the abdomen, hemogram, and bone marrow biopsy were normal. Only one patient received therapy (local radiotherapy) before entering this study.

Histological and Immunohistochemical Evaluations. Sections of formalin-fixed paraflin-embedded biopsies, were stained with hematoxylin & eosin, Giemsa, periodic acid-Schiff, Gomori, and Weigert-Van Gieson for the routine histological diagnosis and classification according to Kiel (15), Rappaport (16), and the Working Formulation (WF) (17).

The immunohistochemical reactions were performed on cryostat sections (5 µm) from skin biopsies (snap-frozen in liquid nitrogen and stored at −80 °C), air dried overnight before fixing in acetone (10 min at room temperature) and stained according to the APAAP immunokaline phosphatase method (18) with commercial reagents (Dako, Glostrup, Denmark). The MoAbs specific for the cluster differentiation CD19 (19), CD22 (20), CD23 (20), and CD21 (21) were obtained from the Second and Third International Workshops on Human Leucocyte Differentiation Antigens. HLA-DR molecules were detected with the MoAb DA2 (22). MoAbs specific for human κ and λ immunoglobulin light chains were purchased from Dako. The MoAbs Ki-M4b (23) and Ki-67 (24), specific for DRC of the follicle and for a proliferation-associated nuclear antigen, respectively, were purchased from Behring (Berlin).

DNA Isolation and Genomic Southern Blot Analysis. High molecular weight DNA was extracted from the same lymphoma biopsies used for the structural analysis of the immunoglobulin genes. In addition, the analysis of the breakpoints of the chromosomal rearrangements has served to identify genes important in inducing the tumor or affecting its progression (10, 11). Furthermore, the development of probes specific for such breakpoints can overcome the difficulties that can sometimes hamper the cytogenetic studies.

Taking advantage of these techniques, we decided to investigate the molecular aspect of primary cutaneous lymphomas of B-cell phenotype to confirm the B-cell lineage and clonality by structural analysis of the immunoglobulin genes. In addition, it was of interest to determine whether the cases presented abnormalities of protooncogenes involved in other B-NHL, such as c-myc, implicated in Burkitt’s lymphomas (12) and bcl-2, a breakpoint region occurring in follicular B-NHL (13), as well as the ß chain of the TCR complex, whose “inappropriate” rearrangement has been detected in some B-cell lymphomas (14).

MATERIALS AND METHODS

Patients. Fourteen patients were included in this study, six females and eight males, median age 57 (range, 26–28), seven of them presenting skin nodules or plaques localized on the back, three on the head, two on the thorax, and two on the legs. Clinical examination revealed involvement of superficial satellite lymph node in two cases while no abnormalities were found in the remaining. The chest X-ray, computerized axial tomography of the abdomen, hemogram, and bone marrow biopsy were normal. Only one patient received therapy (local radiotherapy) before entering this study.

Histological and Immunohistochemical Evaluations. Sections of formalin-fixed paraflin-embedded biopsies, were stained with hematoxylin & eosin, Giemsa, periodic acid-Schiff, Gomori, and Weigert-Van Gieson for the routine histological diagnosis and classification according to Kiel (15), Rappaport (16), and the Working Formulation (WF) (17).

The immunohistochemical reactions were performed on cryostat sections (5 µm) from skin biopsies (snap-frozen in liquid nitrogen and stored at −80 °C), air dried overnight before fixing in acetone (10 min at room temperature) and stained according to the APAAP immunokaline phosphatase method (18) with commercial reagents (Dako, Glostrup, Denmark). The MoAbs specific for the cluster differentiation CD19 (19), CD22 (20), CD23 (20), and CD21 (21) were obtained from the Second and Third International Workshops on Human Leucocyte Differentiation Antigens. HLA-DR molecules were detected with the MoAb DA2 (22). MoAbs specific for human κ and λ immunoglobulin light chains were purchased from Dako. The MoAbs Ki-M4b (23) and Ki-67 (24), specific for DRC of the follicle and for a proliferation-associated nuclear antigen, respectively, were purchased from Behring (Berlin).

DNA Isolation and Genomic Southern Blot Analysis. High molecular weight DNA was extracted from the same lymphoma biopsies used for the immunophenotypic analysis, digested (20 µg) to completion with

Received 6/10/88; revised 1/27/89, 5/18/89; accepted 5/26/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Th e abbreviations used are: B-NHL, B-cell non-Hodgkin’s lymphoma; TCR, T-cell receptor; WF, working formulation; MoAb, monoclonal antibody; CD, cluster differentiation; DRC, dendritic reticulum cell; EBV, Epstein-Barr virus.
RESULTS

Histological and Immunophenotypic Findings. Histological examination of the cases revealed the presence of either follicular or diffuse lymphoid infiltrate formed of large cells of germinal center cell type extending from the papillary dermis to the s.c. tissue; a zone of normal collagen (Grenz zone) separated the dermal infiltrate from the epidermis. The histopathological classification of the cases is reported in Table 1; according to the Working Formulation (WF) six cases were of low grade and eight of intermediate grade malignancy. The immunohistochemical study showed, in all cases, a strong reactivity of the neoplastic cells with the HLA-DR, CD19, and CD22 specific MoAbs (Fig. 1); CD23 was negative in all, except case 1. The T-cell marker CD2 was positive on infiltrating T-cells but not on the neoplastic population (not shown); CD2+ cells varied from 5 to 10% in CbCcD and CdD histotypes, to more than 50% in the follicular areas of CbCcD or at the periphery of the neoplastic nodules. Monoclonal immunoglobulin light chains were clearly demonstrated in six cases (four κ, two λ; Table 1). Nuclear staining for the proliferation-associated molecule identified with the Ki-67 MoAb was seen in all biopsies, localized in 20–40% of the neoplastic infiltrate [as determined using serial tissue sections, as well as by double immunostaining for CD22 (or CD2) and Ki-67].

A network of DRC, identified with the Ki-M4b and CD23 MoAbs (the latter known to react with both B and DRC) was observed within the follicles of cases 1, 2, 4, 5, and 6; in the diffuse sites the DRC were scarce or absent.

These data indicate that the infiltrating neoplastic cells have a B-cell phenotype, are capable of forming follicular structures, and may have a monoclonal origin as suggested by the immunoglobulin light chain restriction, clearly measured in 40% of the cases.

Molecular Analysis. The DNA, extracted from the 14 biopsies in a well-preserved form, was analyzed for rearrangements of the immunoglobulin heavy and light chains, bcl-2, c-myc, TCR β, and TCR γ genes, and for the presence of EBV. The results are listed in Table 2.

It must be noted that the variable amount of normal tissue (stratified epithelia, adnexa, vessels, fibroblasts) affected the ratio normal/tumor DNA of each individual biopsy; this value cannot be inferred from the immunohistochemical data reported in Table 1, which are referred to the total amount of infiltrating cells). With the JH probe used for immunoglobulin heavy chain gene (which generates germ-line bands of 18.0 and 9.5 kilobases on DNA digested with BamHI and HindIII, respectively) mononclonal rearrangement bands, involving one or both alleles, were found in 12 of 14 cases. In all cases germ-line bands were observed indicating the presence of nonclonal cells (Fig. 2, A and B).

The presence of a faint band of 12 kilobases (in addition to the germ line) was seen in BamHI digests of all samples including the control; this phantom J band has been previously observed within the follicles of cases 1, 2, 4, 5, and 6; in the diffuse sites the DRC were scarce or absent.

These data indicate that the infiltrating neoplastic cells have a B-cell phenotype, are capable of forming follicular structures, and may have a monoclonal origin as suggested by the immunoglobulin light chain restriction, clearly measured in 40% of the cases.

Molecular Analysis. The DNA, extracted from the 14 biopsies in a well-preserved form, was analyzed for rearrangements of the immunoglobulin heavy and light chains, bcl-2, c-myc, TCR β, and TCR γ genes, and for the presence of EBV. The results are listed in Table 2.

It must be noted that the variable amount of normal tissue (stratified epithelia, adnexa, vessels, fibroblasts) affected the ratio normal/tumor DNA of each individual biopsy; this value cannot be inferred from the immunohistochemical data reported in Table 1, which are referred to the total amount of infiltrating cells). With the JH probe used for immunoglobulin heavy chain gene (which generates germ-line bands of 18.0 and 9.5 kilobases on DNA digested with BamHI and HindIII, respectively) mononclonal rearrangement bands, involving one or both alleles, were found in 12 of 14 cases. In all cases germ-line bands were observed indicating the presence of nonclonal cells (Fig. 2, A and B).

The presence of a faint band of 12 kilobases (in addition to the germ line) was seen in BamHI digests of all samples including the control; this phantom J band has been previously reported.

Monoclonal immunoglobulin light chain rearrangements were detected in all samples (nine for κ, four for λ, and one for

Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>Immunophenotype</th>
<th>Surface markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + - K</td>
</tr>
<tr>
<td>2</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + - + -</td>
</tr>
<tr>
<td>3</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>4</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>5</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>6</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>7</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>8</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>9</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>10</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>11</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>12</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>13</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>14</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
</tbody>
</table>

a The abbreviations used are: CbCcFD, centroblastic-centrocytic follicular and diffuse; CbCcD, centroblastic-centrocytic diffuse; CdD, centroblastic diffuse.

b NMLH, nodular, mixed lymphocytic and histiocytic; DH, diffuse histiocytic; DMLH, diffuse, mixed lymphocytic and histiocytic; DHPD, diffuse histiocytic, poorly differentiated.

c The abbreviations used are: CbCcFD, centroblastic-centrocytic follicular and diffuse; CbCcD, centroblastic-centrocytic diffuse; CdD, centroblastic diffuse.

The presence of a faint band of 12 kilobases (in addition to the germ line) was seen in BamHI digests of all samples including the control; this phantom J band has been previously reported.

Monoclonal immunoglobulin light chain rearrangements were detected in all samples (nine for κ, four for λ, and one for

Table 2

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>Immunophenotype</th>
<th>Surface markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + - K</td>
</tr>
<tr>
<td>2</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + - + -</td>
</tr>
<tr>
<td>3</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>4</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>5</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>6</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>7</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>8</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>9</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>10</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>11</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>12</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>13</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>14</td>
<td>CbCcFD</td>
<td>NMLH</td>
<td>+ + + + + -</td>
</tr>
</tbody>
</table>

a The abbreviations used are: CbCcFD, centroblastic-centrocytic follicular and diffuse; CbCcD, centroblastic-centrocytic diffuse; CdD, centroblastic diffuse.
**Fig 1.** Immunostaining of cryostatic skin biopsy sections with B-cell, DRC, and proliferation markers. In a, two CD19-positive follicular structures (*) are shown (184 x). On serial sections the same field (742 x), stained for CD19 (b), contains Kim-4b reactive DRC (arrows; c) and nuclei expressing the proliferation-associated antigen Ki-67 (d).

**Fig 2.** Southern blot analysis of immunoglobulin genes. DNA from lymphoma biopsies and from granulocytes (used as germ-line control) was digested with HindIII (A), BamHI (B, C) or EcoRI (D) and hybridized with JH (A and B), CK (C) and JL (D). The numbers indicated correspond to the patients reported in the tables. Germ-line and rearrangement bands are indicated by dashes and arrows, respectively. Molecular weights in kilobases. Note: the faint common extra band of 12 kilobases obtained with the JH probe (A) is due to a phantom J band, as observed by other authors (38).

Both (using CK and JL) as probes, thus including cases 1 and 7, which were negative for JH (Fig. 2, C and D). This finding was further investigated to verify the possibility that the unexpected extra bands seen in the CK probe were due to a polymorphism; thus, HindIII digests from these two cases were hybridized to a JK probe; the results showed that whereas case 1 was also rearranged for JK (both alleles) case 7 presented only the 5.0-kilobase germ-line band (data not shown).

Deletions of the λ light chain gene were not evident in the cases rearranged for λ. Because the κ gene is rearranged or deleted in B-cells rearranged for λ (38), it is possible that the κ light chain gene is only apparently in germ-line configuration. In fact, in samples with a relatively low percentage of tumor cells, whereas rearrangements are still detectable, deletions can be missed.

The bcl-2 region, studied with the probes pFL1 and pFL2, specific for the two cluster regions of the t(14;18) breakpoint, was in germ-line configuration (Fig. 3, A and B), indicating that this translocation is not involved.

Structural alterations or amplifications of the c-myc oncogene were not present in any of the samples (Fig. 3C). Alterations of the TCRβ and TCRγ genes were not observed (Fig. 3, D and E) indicating the absence of clonal T-cells and of “inappropriate rearrangements,” occasionally observed in nodal B-NHL. The DNA from all specimens was tested for the presence of EBV sequences and gave negative results (Table 2).

**DISCUSSION**

Only recently the occurrence of primary cutaneous B-cell lymphomas of germinal center cell origin, diagnosed with immunological methods, has been reported in the literature (5–7).

Immunohistological data presented here extend these previous results and clearly show, using well-characterized monoclonal antibodies, that the neoplastic cells belong to the B-cell lineage since they react with the B-cell markers CD19 and CD22 but not with the T-cell marker CD2 and unequivocally...
present, in some cases, monoclonal immunoglobulin light chains.

Moreover, the finding that none of the cases, except one, expresses CD23, an antigen found on follicular mantle B-cells and, occasionally, on a fraction of germinal center cells of lymph nodes (40), suggests that these cutaneous lymphomas arise from the malignant transformation of cells of follicular center type. This evidence, consistent with the morphology, is also supported by the observation that CD1c, a marker of mantle zone but not germinal center B-cells (41), is negative in our cases. The "in situ" proliferative capacity of the neoplastic B-cells was demonstrated, on serial tissue sections, by Ki-67, a MoAb already employed to measure the tumour-cell growth fraction of nodal NHL, a parameter which positively correlates with the histological grade (42) and prognosis (43). Although we have used the Ki-67 marker to document the proliferative activity of the neoplastic infiltrate, studies are under progress to evaluate its prognostic significance.

The existence of true follicular structures in some CbCcFD histotypes was shown using the DRC-specific MoAb Kim4b (23); the intimate association between accessory cells and neoplastic B-cells of germinal center origin has been observed in various nodal and extranodal lymphomas (44).

This study was also undertaken to confirm, by molecular genetic analysis, the clonality and lineage of these phenotypically defined lymphomas and to determine whether two proto-oncogenes frequently associated with B-NHL, namely c-myc and bcl-2, are involved in the pathogenesis of this neoplasm.

By using random primer radiolabeled probes we have demonstrated that 85% of the cases have monoclonal IgH rearrangements, involving both alleles in 16.5% of cases, and immunoglobulin light chain monoclonal bands clearly confirmed in 13 cases. These results, together with the finding that neither TCRβ nor TCRγ rearranged bands were seen, indicate that the disease is clonal and of B-cell origin.

The finding, in cases 1 and 7, of rearranged immunoglobulin light chain without apparent rearrangement of the heavy chain gene (and despite κ light chain monoclonal expression in case 1) is rather unusual, though occasionally observed (45, 46). One possibility is that the κ chain gene band arises from a polymorphism rather than from a physiological rearrangement; the results excluded this possibility in case 1 but not in case 7 since the κ rearrangements were confirmed with two different probes and enzymes in the former and only with the CK probe in the latter.

However, the recent identification of pre-B-cell clones (derived from EBV-infected normal fetal B-cell progenitors) displaying κ light chains but not heavy chain gene rearrangements and expressing membrane κ light chain (but not heavy chain) immunoglobulin (47) suggests the existence of a new pathway of B-cell development. It is conceivable that at least case 1 might represent clonal expansion of this genotypically unusual category of cells.

The interest in assessing the molecular structure of the bcl-2 oncogene; this was actually not surprising since, so far, it has been found rearranged in only high grade B-cell malignancies such as the Burkitt's lymphomas (12), lymphoblastic leukemias (4), and large cell lymphomas (50).

In summary, this study, aimed at elucidating the phenotypic and molecular features of the primary cutaneous lymphoma, shows that the neoplastic infiltrate has a B-cell composite phenotype compatible with that of follicular center cells; rearrangements of immunoglobulin heavy and light chains but neither TCRβ nor TCRγ indicate that the disease is monoclonal, of B-cell origin (as demonstrated in 13 of 14 cases) and with no "inappropriate rearrangements." Since no structural alterations of the c-myc and bcl-2 proto-oncogenes or EBV sequences have been found, it appears that the pathogenetic features of this disease remain to be further investigated.

REFERENCES


* E. Berti, unpublished data.


Clonal Immunoglobulin Gene Rearrangements and Normal T-Cell Receptor, bcl-2, and c-myc Genes in Primary Cutaneous B-Cell Lymphomas

Domenico Delia, Maria G. Borrello, Emilio Berti, et al.


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/49/17/4901

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.