KAPOSI'S SARCOMA

DAVID J. LOONEY, MD

Dr Looney was invited by the IAS-USA to select the key clinical and scientific findings presented or published in the last year on Kaposi's sarcoma and to provide a commentary on the current status of this treatment area. Dr Looney is Assistant Professor of Medicine at the University of California San Diego and the Department of Veterans Affairs Medical Center, San Diego, California.

SELECT STUDIES

Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N Engl J Med.* 1995;332:1181–1185.

This paper expands upon the elegant Science paper[1] published in late 1994 that described the use of representational difference analysis to detect gammaherpesvirus sequences in Kaposi's sarcoma (KS) lesions. Using a polymerase chain reaction (PCR) technique, investigators analyzed DNA in tissue samples from patients with AIDS-KS, those with classic KS, and HIV-1-seronegative homosexual males with KS. The analysis demonstrated a very close association between the detection of human herpesvirus type 8 (HHV-8) and the histologic presence of KS, and confirmed the paucity of HHV-8 sequences in (unmatched) control specimens.

Moore PS, Gao SJ,Dominguez G, et al. primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J. Virol.* 1996;70:549-558.

This article presents a further characterization of the molecular structure of HHV-8 and analysis of serological reactivity. The introduction of a serological (indirect immunofluorescence) assay for detection and quantitation of antibodies to HHV-8 should pave the may to more meaningful epidemiologic studies. Interesting homologies noted in 18 sequenced open reading frames of this agent with cellular and other viral genes suggest intriguing directions for further research. Finally, data presented on induction of viral replication in cell lines with phorbol esters (TPA) may have served to guide other researchers attempting to culture this virus in vitro (see below).

Renne R, Zhong WD, Herndier B, et al. Lytic growth of Kaposis sarcoma-associated herpesvirus (human herpesvirus in culture. *Nature Med.* 1996;2:342-346.

These investigators conclusively demonstrate that the gammaherpesvirus-like sequences found in Kaposi's sarcoma belong to a replicating virus, and provide the first electron micrographs of the virus. The availability of systems for studying HHV-8 replication will allow the characterization of activity of a number of anti-herpesvirus agents against HHV-8, potentially providing important information for the development of trials of antivirals in treatment of Kaposi's sarcoma.

Lunardi-Iskandar Y, Bryant JL, Zeman RA, et al. Tumorigenesis and metastasis of neoplastic Kaposi's sarcoma cell line in immunodeficient mice blocked by a human pregnancy hormone. *Nature.* 1995;375:64–68.

This paper, together with a paper describing the isolation and characterization of this tumorigenic KS cell line published by the same authors a few months later,[2] represent a second new alternative hypothesis of the origin of KS. The authors describe an aneuploid cell line (KS Y-1) derived from KS that is capable of tumorigenesis and metastasis in a murine model; they use this model to demonstrate the inhibitory activity of the beta subunit of human chorionic gonadotropin, which has led to ongoing clinical trials (and a number of letters to journal editors describing anecdotal responses to human chorionic gonadotropin in humans).

Popescu NC, Zimonjic DB, Leventon-Kriss S, et al. Deletion and translocation involving chromosome 3(p14) in two tumorigenic Kaposi's sarcoma cell lines. *J Natl Cancer Inst.* 1996;88:450-455.

Investigators describe chromosomal lesions typical of two aneuploid tumor cells lines derived from KS. The identification of specific deletions and translocations in cell lines allows examination of tissue from lesions using PCR and other more sensitive modalities to be performed. Determination of the prevalence of cells bearing such genetic lesions in different stages of the disease may lead to a better definition of the role of such cells in KS oncogenesis.

Fiorelli V, Gendelman R, Samaniego F, Markham PD, Ensoli B. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. *J Clin Invest.* 1995;95:1723–1734.

Appearing the same month as an article by this National Cancer Institute group in the *Journal of Immunology*[3], this paper extends upon previously published work that supports yet other distinct mechanisms possibly responsible for the proliferation of spindle cells and development of KS lesions, including the proliferative effects of HIV-1 Tat protein and basic fibroblast growth factor.[4,5] These data are consistent with the theory that both immunosuppression and immunostimulation are required for the development of KS, illustrating that the phenotype and biologic behavior of activated endothelial cells and cell lines derived from KS lesions are virtually indistinguishable. In this respect, the data may be too convincing, suggesting to the reader that "Kaposi's" cells studied in vitro represent only activated endothelial cells, while leaving unexplained their distribution and genesis.

Rabkin CS, Bedi G, Musaba E, et al. AIDS-related Kaposi's sarcoma is a clonal neoplasm. *Clin Cancer Res.* 1995;1:257–260.

Providing yet another piece of the KS puzzle is this first and long-awaited report of the clonality of KS lesions. Using PCR to amplify a fragment of the human androgen receptor gene (X chromosome) containing a methylation restriction

site on tissues from KS lesions from female patients, the investigators detected monoclonal cell populations in two lesions and clonal proliferations in a third. It may be difficult to reconcile such evidence of clonality (reflecting the bulk of cells in the lesion examined) with the existence of only a small population of "true" malignant cells in lesions.

Harrison M, Tomlinson D, Stewart S. Liposomal-entrapped doxorubicin: an active agent in AIDS-related Kaposi's sarcoma. *J Clin Oncol.* 1995;13:914–920.

Although this article may be properly viewed as just one of more than 20 articles on the use of liposome-entrapped doxorubicin and daunorubicin[6,7] appearing over the past 3 years, it is noteworthy for representing Doxil™, recently approved by the FDA, as an emerging standard for the treatment of AIDS-related KS. In addition, this article illustrates the general emphasis on identifying reasonably effective, well-tolerated treatments. In this phase II study, thirty-four patients with AIDS-related KS (median Karnofsky score 70) were treated with 20 mg/m^2 of liposome-entrapped doxorubicin every three weeks. The overall response rate was 73.5% (25/34 patients); in patients who had received prior chemotherapy, the response rate was 68.4% (13/19 patients). In terms of side effects, neutropenia, alopecia, nausea and vomiting occurred in 34%, 9%, and 18% of patients, respectively.

Paredes J, Kahn JO, Tong WP, et al. Weekly oral etoposide in patients with Kaposi's sarcoma associated with human immunodeficiency virus infection: a phase I multicenter trial of the AIDS Clinical Trials Group. *J Acq Immun Def Syn Human Retrovirol.* 1995;9:138–144.

Etoposide has been used successfully for treatment of KS. In this dose-ranging study (150 to 400 mg/week), oral etoposide resulted in a partial response rate of 36% (9/25). The agent was well tolerated at lower doses, suggesting another useful, minimally toxic, therapeutic alternative in patients with AIDS and KS.

Saville MW, Lietzau J, Pluda JM, et al. Treatment of HIV-associated Kaposi's sarcoma with paclitaxel. *Lancet.* 1995;346:26–28.

This paper is the first of several that can be expected on the use of taxol in KS. Use of paclitaxel as a single agent (135 mg/m^2 IV over 3 hours every 21 days) produced a partial response rate of 65% (13/20 patients), a result comparable to the best overall response rates obtained with liposomal anthracyclines. In addition, paclitaxel was associated with acceptable toxicity (most frequently neutropenia, and some unexpected rash and eosinophilia).

COMMENTARY

Kaposi's sarcoma (KS) is a multicentric, highly vascular, proliferative disorder involving endothelial cells, fibroblasts, and characteristic spindle-shaped mesenchymal cells. HIV-1 infection represents an overwhelming risk factor (20,000:1)[8] for the development of KS, which was a rare tumor in the United States (incidence less than 1/100,000/year) before the HIV-1 epidemic. Today, KS remains the most frequent neoplasm affecting HIV-infected individuals and a cause of substantial morbidity and mortality in AIDS patients. Approximately 25% to 30% of homosexual males with HIV infection develop clinically significant KS, and autopsy studies[9-11] indicate an even higher prevalence of the disease (up to approximately 40%). Aggressive disease, with involvement of the gut, lung, pleura, and lymph nodes, is seen frequently in AIDS-related KS,[10] as is a predilection for lesions of the hard and soft palates.

The etiology of KS, the reasons for the increased incidence of KS in AIDS patients, and the explanation for the marked predilection of KS for the male sex (greater than 15:1 male:female overall, approximately 4:1 male:female in AIDS patients when homosexual males are excluded) have been topics of considerable controversy over the last 2 years. Although KS is associated with immunosuppression due to other conditions, such as renal transplant, chronic lymphocytic leukemia, mycosis fungoides, multiple myeloma, and thymoma, this does not entirely explain the excess of KS seen in HIV disease. Similarly, a rational explanation for the marked predilection of KS for the male sex is still lacking, although a role for the direct or indirect effect of gonadotropic hormones is an intriguing hypothesis (see below).

Epidemiologic evidence has suggested that a transmissible infectious agent might be involved in KS,[8] but only recently have molecular techniques permitted Moore and Chang, and others,[1,12-14] to identify a novel gammaherpesvirus ("Kaposi's sarcoma herpesvirus" [KSHV], now referred to as human herpesvirus type 8 [HHV-8]) as a likely candidate. The association of HHV-8 with KS, now well established by a number of papers,[11,12] does not suffice to establish a causal role for this virus. Note that Moore and Chang, and Chang and colleagues[1] detected HHV-8 sequences in 21% of "matched" control samples (uninvolved skin from KS patients), raising doubts concerning the specificity of the association between virus and lesions in patients known to be positive for the virus. Others have noted that HHV-8 sequences frequently are present in non-KS lesions.[14] Other preliminary results suggest a close relationship of HHV-8 with Epstein-Barr virus (EBV), with EBV frequently being detected in KS tissue but not other tissues from individuals with KS.[15] Other possible explanations for the close association of HHV-8 with KS include a tropism for virus replication in activated endothelial cells or other cell types in KS tumors,[16] or localization in KS lesions due to attachment ("filtration") of circulating B-cells harboring latent virus[17] to activated endothelial-cell adhesion molecules. The development of methods to allow culture, replication, and passage of HHV-8 virus by Ganem's group (Renne R, et al), and similar work being presented by G. Nabel, J.A. Levy and others, together with serological methods for epidemiology should allow rapid determination of prevalance of HHV-8 infection, and shed additional light on causality, and the usefulness of antiviral treatment (see below).

A number of letters have followed a 1994 article[18] describing anecdotal responses of KS to treatment with antiviral agents, such as foscarnet sodium. Even if a transforming role for HHV-8 is established in KS, it is not clear that inhibition of virus replication would affect tumor growth. It is also possible that foscarnet, having activity against HIV, may have produced a response through action on underlying HIV disease. This appears not unlikely in view of the known activity of zidovudine in

early disease[19,20] and recent reports (David Cooper, Second National Conference on Human Retroviruses and Related Infections, January 29–February 2, 1995, Washington, DC) of regression of KS in patients treated with HIV protease inhibitors.

It becomes more difficult to visualize a role for treatment of herpesvirus in KS when considering the recent isolation of aneuploid transformed cells from KS lesions by Lunardi-Iskander and colleagues. Although spindle-shaped diploid cells (KS-cells) morphologically similar to those seen in KS lesions have been cultured[21-23] from lesions, effusions, and peripheral blood in vitro, these cells have not been found to harbor identifiable chromosomal abnormalities and are neither immortalized nor transformed. Rather, Fiorelli and colleagues and others[3-5,24,25] found that the cells require growth factors supplied by activated lymphoid cells, and they may be virtually indistinguishable from activated microvascular endothelial cells.

The use of these new tumorigenic KS cell lines in murine models has led to identification of new candidate therapeutic agents. A number of letters have appeared concerning anecdotal treatment with human chorionic gonadotropin,[26] and trials are in progress. In any case, the use of models with tumorigenic KS cell lines is leading to testable hypotheses, wherein activity or lack of activity in murine models can be compared with findings in human trials.

Although it offers a potential explanation for the paucity of KS in female patients, the predominance of normal diploid cells in KS lesions presents a significant problem to proponents of genetically abnormal, malignant, transformed cell type as a principal cause of KS. The recent data cited by Rabkin and colleagues suggesting clonality of cells within KS lesions makes this all the more difficult to explain, since the techniques used are quantitative, rather than qualitative, indicating a clonal expansion of the bulk of the cells comprising the lesion tissue. The mechanism whereby the "true" tumor cell would induce clonal proliferation in normal surrounding cells is not clear, and if the aneuploid cells themselves are not responsible for the clinical manifestations of the disease, they may not represent the only or the ideal target for treatment.

Fiorelli and colleagues, and others,[4,5,23-25] have shown a variety of cytokines to be active in the maintenance proliferation of normal diploid KS cells in vitro. It is not surprising that cytokine inhibitors[27] and other inhibitors of angiogenesis—including thalidomide, metalloproteinase inhibitors, thrombospondin analogues, apoliproprotein E, integrin antagonists, fumagillin (TNP-470), heparin-steroid conjugates, CM101, SP-PG, and pentosan sulfate[28-36]—are being pursued as potential therapeutic agents for KS. To date, however, the results from the use of such polycationic angiogenesis inhibitors as pentosan sulfate and suramin have not been promising.[37] Similarly, results[38] from initial trials with differentiating agents such as all-*trans* retinoic acid have fallen short of expectations; however, accumulating evidence from multicenter trials and a number of abstracts submitted to upcoming meetings indicate that topical treatment with retinoids is moderately effective, and the availability of a plethora of retinoid analogues suggests that additional exploration may be indicated.

Conventional treatments for KS are generally considered to be palliative, including localized radiation therapy, immunotherapy and antiviral therapy with zidovudine and alpha interferon, intralesional therapy with a variety of agents, and the use of a number of different conventional cytotoxic chemotherapy agents alone or in combination (including vinblastine, topoisomerase II inhibitors, and anthracyclines). Over the past few years, intensive combination chemotherapy has been largely abandoned, with an emphasis being placed on finding therapeutic compromises between efficacy and toxicity in treating KS.

As noted in the paper by Harrison and colleagues, and by others,[6,7] the use of liposomal preparations of anthracycline drugs as single agents has represented one such emerging compromise. These agents can be administered with relative safety and create little degradation in quality of life for the patient. The availability of granulocyte colony-stimulating factor, to prevent or reduce the principal complication of neutropenia, has also made administration of marrow-suppressive agents to already immunocompromised individuals a less frightening proposition. In addition, the use of oral etoposide and the introduction of taxol represent other attractive treatment alternatives that are likely to see increasing use over the next few years.

In summary, previous efforts to explain the origin of KS have centered on the study of the interaction of host immunity, host immune stimulation, and viral factors, such as the HIV-1 Tat protein. A complex web of growth factors and cytokines has been found to play a role in proliferation of cell lines derived from KS cells in vitro. Two alternative, but not necessarily mutually exclusive, schemas for KS oncogenesis—direct or indirect viral oncogenesis and involvement of "true" malignant cells—were identified in 1995. Novel treatment strategies suggested by all three hypotheses are being explored, including angiogenesis inhibition, antiviral treatment, and hormonal manipulation. In addition, conventional treatment regimens have continued to evolve, with an emphasis on identifying reasonably effective, minimally toxic therapies. The identification of an increasing number of molecules involved in angiogenesis (and inhibitors of these angiogenic moieties), the ability to cure the associated herpesvirus and identify active viral compounds, together with availability of new cell lines and new animal models for testing, indicate promising prospects for the year to come.

REFERENCES

1. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science.* 1994;266:1865–1869.
2. Lunardi-Iskandar Y, Gill P, Lam VH, et al. Isolation and characterization of an immortal neoplastic cell line (KS Y-1) from AIDS-associated Kaposi's sarcoma. *J Natl Cancer Inst.* 1995;87:974–981.
3. Samaniego F, Markham PD, Gallo RC, Ensoli B. Inflammatory cytokines induce AIDS-Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. *J Immunol.* 1995;154:3582–3592.
4. Albini A, Barillari G, Benelli R, Gallo RC, Ensoli B. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. *Proc Natl Acad Sci USA* 1995;92:4838–4842.
5. Ensoli B, Gendelman R, Markham P, et al. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature.* 1994;371:674–680.
6. Bogner JR, Kronawitter U, Rolinski B, et al. Liposomal doxorubicin in the treatment of advanced aids-related Kaposi sarcoma. *J Acq Immun Def Syn Human Retrovirol.* 1994;7:463-468.
7. Gill PS, Espina BM, Muggia F, et al. Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. *J Clin Oncol.* 1995;13:996–1003.

8. Beral V, Peterman TA, Berkelman RL, Jaffe HW. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet.* 1990;335:123–127.
9. Safai B, Johnson KG, Myskowski PL, et al The natural history of Kaposi's sarcoma in the acquired immunodeficiency syndrome. *Ann Intern Med.* 1985;103:744–750.
10. Klatt EC, Nichols L, Noguchi TT. Evolving trends revealed by autopsies of patients with the acquired immunodeficiency syndrome. 565 autopsies in adults with the acquired immunodeficiency syndrome, Los Angeles, California, 1992–1993. *Arch Pathol Lab Med.* 1994;118:884–990.
11. Katz MH, Hessol NA, Buchbinder SP, et al. Temporal trends of opportunistic infections and malignancies in homosexual men with AIDS. *J Infect Dis.* 1994;170:198–202.
12. Huang YQ, Li JJ, Kaplan MH, et al. Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. *Lancet.* 1995;345:759–61.
13. Whitby D, Howard MR, Tenant-Flowers M, et al. Detection of Kaposi's sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet.* 1995;346:799–802.
14. Rady PL, Yen A, Rollefson JL, et al. Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. *Lancet.* 1995;345:1339–1340.
15. Corbeil J, Badel P, Baird S, Shin S, Looney DJ. Frequency of KSHV, EBV, and HHV6 sequences in Kaposi's sarcoma tissue, PBMC, cell lines, and inflammatory vascular lesions. Abstracts of the Tumor Cell Biology Laboratory Meeting, Bethesda, Md, 1995.
16. Boshoff C, Schulz TF, Kennedy MM, et al. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nature Med.* 1995;1:1274–1283.
17. Ambroziak JA, Blackbourn DJ, Herndier BG, et al. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients (letter). *Science.* 1995;268:582–3.
18. Morfeldt L, Torssander J. Long-term remission of Kaposi's sarcoma following foscarnet treatment in HIV-infected patients. *Scand J Infect Dis.* 1994;26:749–52.
19. Kovacs JA, Deyton L, Davey R, et al Combined zidovudine and interferon-alpha therapy in patients with Kaposi's sarcoma and the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med.* 1989;111:280–287.
20. Krown SE, Gold JW, Niedwiecki D, et al Interferon-alpha with zidovudine—safety, tolerance, and clinical and virologic effects in patients with Kaposi's sarcoma associated with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med.* 1990;112:812–821.
21. Salahuddin SZ, Nakamura S, Biberfeld P, et al. Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture in vitro. *Science.* 1988;242:430–433.
22. Ensoli B, Nakamura S, Salahuddin SZ, et al. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science.* 1989;243:223–226.
23. Corbeil J, Evans LA, Vasak E, Cooper DA, Penny R. Culture and properties of cells derived from Kaposi's sarcoma. *J Immunol.* 1991;146:2972–2976.
24. Miles SA, Rezai AR, Salazar-Gonzalez JF, et al. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc Natl Acad Sci USA.* 1990;87:4068–4072.
25. Sturzl M, Brandstetter H, Zietz C, et al. Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined in vitro and in vivo analysis. *Oncogene.* 1995;10:2007-2016.
26. McNamee D. Beta-hCG inhibits Kaposi's sarcoma (news). *Lancet.* 1995;345:1169.
27. Louie S, Cai J, Law R, et al. Effects of interleukin-1 and interleukin-1 receptor antagonist in AIDS-Kaposi's sarcoma. *J Acq Immun Def Syn Human Retrovirol.* 1995;8:455-460.
28. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA.* 1994;91:4082-4085.
29. Dameron KM, Volpert OV, Tainsky MA, Bouck N. Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science.* 1994;265:1582-1584.
30. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell.* 1994;79:1157-1164.
31. Browning PJ, Roberts DD, Zabrenetzky V, et al. Apolipoprotein E (ApoE), a novel heparin-binding protein inhibits the development of Kaposi's sarcoma-like lesions in BALB/c nu/nu mice. *J Exp Med.* 1994;180:1949-1954.
32. Quinn TE, Thurman GB, Sundell AK, Zhang M, Hellerqvist CG. CM101, a polysaccharide antitumor agent, does not inhibit wound healing in murine models. *J Cancer Res Clin Oncol.* 1995;121:253-256.
33. Tanaka H, Taniguchi H, Mugitani T, et al. Intra-arterial administration of the angiogenesis inhibitor TNP-470 blocks liver metastasis in a rabbit model. *Br J Cancer.* 1995;72:650-653.
34. Thorpe PE, Derbyshire EJ, Andrade SP, et al. Heparin-steroid conjugates: new angiogenesis inhibitors with antitumor activity in mice. *Cancer Res.* 1993;53:3000–3007.
35. Nakamura S, Sakurada S, Salahuddin SZ, et al. Inhibition of development of Kaposi's sarcoma-related lesions by a bacterial cell wall complex. *Science.* 1992;255:1437–1440.
36. Benelli R, Adatia R, Ensoli B, et al. Inhibition of AIDS-Kaposi's sarcoma cell induced endothelial cell invasion by TIMP-2 and a synthetic peptide from the metalloproteinasepropeptide: implications for an anti-angiogenic therapy. *Oncol Res.* 1994;6:251–257.
37. Pluda JM, Shay LE, Foli A, et al. Administration of pentosan polysulfate to patients with human immunodeficiency virus-associated Kaposi's sarcoma. *J Natl Cancer Inst.* 1993;85:1585–1592.
38. Bailey J, Pluda JM, Foli A, et al. Phase I/II study of intermittent all-trans-retinoic acid, alone and in combination with interferon alfa-2a, in patients with epidemic Kaposi's sarcoma. *J Clin Oncol.* 1995;13:1966–1974.