

ORIGINAL ARTICLE

Sirolimus for Kaposi's Sarcoma in Renal-Transplant Recipients

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ABSTRACT

BACKGROUND

Recipients of organ transplants are susceptible to Kaposi's sarcoma as a result of treatment with immunosuppressive drugs. Sirolimus (rapamycin), an immunosuppressive drug, may also have antitumor effects.

METHODS

We stopped cyclosporine therapy in 15 kidney-transplant recipients who had biopsy-proven Kaposi's sarcoma and began sirolimus therapy. All patients underwent an excisional biopsy of the lesion and one biopsy of normal skin at the time of diagnosis. A second biopsy was performed at the site of a previous Kaposi's sarcoma lesion six months after sirolimus therapy was begun. We examined biopsy specimens for vascular endothelial growth factor (VEGF), Flk-1/KDR protein, and phosphorylated Akt and p70S6 kinase, two enzymes in the signaling pathway targeted by sirolimus.

RESULTS

Three months after sirolimus therapy was begun, all cutaneous Kaposi's sarcoma lesions had disappeared in all patients. Remission was confirmed histologically in all patients six months after sirolimus therapy was begun. There were no acute episodes of rejection or changes in kidney-graft function. Levels of Flk-1/KDR and phosphorylated Akt and p70S6 kinase were increased in Kaposi's sarcoma cells. The expression of VEGF was increased in Kaposi's sarcoma cells and even more so in normal skin cells around the Kaposi's sarcoma lesions.

CONCLUSIONS

Sirolimus inhibits the progression of dermal Kaposi's sarcoma in kidney-transplant recipients while providing effective immunosuppression.

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THE INCIDENCE OF KAPOSI'S SARCOMA among recipients of solid organs is about 500 times the rate in the general population, and among men with the acquired immunodeficiency syndrome (AIDS) it is up to 20,000 times the rate in the general population, suggesting a role for immunosuppression in the development of the disease.¹ Although the overall incidence of Kaposi's sarcoma was 6 percent among graft recipients in the Cincinnati Transplant Tumor Registry, it was 3 percent among azathioprine-treated patients and rose to 10 percent after the introduction of cyclosporine.² The clinical presentation of Kaposi's sarcoma in transplant recipients is often limited to the skin, although visceral Kaposi's sarcoma has been described. The risk of death from Kaposi's sarcoma is related to the form and extent of the lesions at presentation. The main approach to managing transplant-associated Kaposi's sarcoma is to reduce or even discontinue immunosuppressive therapy; this strategy usually causes skin lesions to regress, although it carries a risk of acute rejection of the graft. Kaposi's sarcoma generally recurs when immunosuppressive therapy is reintroduced or after a second transplantation.³

Kaposi's sarcoma is a multicentric tumor composed of endothelium-lined vascular spaces and spindle-shaped cells. Its pathogenesis is unclear, although human herpesvirus 8 (HHV-8) has been implicated.⁴ Recent evidence suggests that this virus up-regulates the vascular endothelial growth factor (VEGF) receptor Flk-1/KDR in endothelial cells.⁵ Indeed, *in vitro* infection of human primary endothelial cells with HHV-8 causes long-term proliferation and survival of the cells. In addition, VEGF is likely a growth factor for Kaposi's sarcoma cells: blocking the interaction between VEGF and Flk-1/KDR can abolish VEGF-induced growth of the tumor.⁶

Sirolimus (rapamycin), an immunosuppressive drug used in kidney-transplant recipients, probably has antineoplastic effects. The immunosuppressive and antineoplastic effects of sirolimus may be due to a common mechanism. Sirolimus inhibits its molecular target (the mammalian target of sirolimus, or mTOR), which links mitogen-induced stimulation of protein synthesis and cell-cycle progression by activating p70S6 kinase, a key enzyme in regulating gene translation.⁷ Inhibition of mTOR prevents acute graft rejection by inhibiting the interleukin-2-induced proliferation of T cells and could block tumorigenesis and metastatic progres-

sion by directly inhibiting the proliferation of tumor cells. Sirolimus inhibits the growth of many tumor cell lines *in vitro* and has antitumor activity in murine tumor models.⁸ Recently, Sodhi et al.⁹ demonstrated that Akt, a serine- or threonine-specific protein kinase directly upstream of mTOR in the sirolimus-sensitive signaling pathway, is activated in Kaposi's sarcoma and is pivotal in the development of Kaposi's sarcoma. Sirolimus also affects angiogenesis in tumors. Guba et al.¹⁰ demonstrated in a mouse model that sirolimus inhibits tumor progression through antiangiogenic activity related to impaired production of VEGF and a limited proliferative response of endothelial cells to stimulation by VEGF. Luan et al. reported similar findings in a mouse model of metastatic renal carcinoma.¹¹ On the basis of these findings, we investigated the cellular and clinical effect of sirolimus on Kaposi's sarcoma in renal-transplant recipients.

METHODS

PATIENTS

Fifteen recipients of a cadaveric kidney transplant who had biopsy-proven Kaposi's sarcoma were enrolled in the study, which ran from October 2001 to March 2004. All patients were tested for human immunodeficiency virus before transplantation, at the time of the diagnosis of Kaposi's sarcoma, and six months later (at the time of the second biopsy), and the results were negative. All patients provided written informed consent. The study was approved by the local ethics committee.

All patients received 500 mg of methylprednisolone intraoperatively, followed by 250 mg of prednisone daily, with the dose tapered to 25 mg by day 8; 20 mg of a chimeric monoclonal antibody against CD25 (Simulect, Novartis) intravenously on day 0 and day 4; and 1 g of mycophenolate mofetil (CellCept, Roche) twice daily. To maintain immunosuppression, they received cyclosporine (Neoral, Novartis, in a dose that kept blood C2 levels in the range of 550 to 750 ng per milliliter), 5 mg of prednisone per day, and 500 mg of mycophenolate mofetil twice daily. When Kaposi's sarcoma was diagnosed, cyclosporine and mycophenolate mofetil were stopped and sirolimus (Rapamune, Wyeth-Ayerst) was started (the loading dose was 0.15 mg per kilogram of body weight, followed by a dose of 0.04 to 0.06 mg per kilogram per day, in order to maintain trough blood levels of 6 to 10 ng per milliliter).

MICROSCOPY

All patients underwent an excisional biopsy of one lesion and one biopsy of normal skin at the time of the diagnosis of Kaposi's sarcoma. A second biopsy was performed six months after the start of sirolimus therapy at the site of a previous Kaposi's sarcoma lesion, to confirm tumor regression histologically. Biopsy specimens were fixed in 4 percent formaldehyde and embedded in paraffin according to standard procedures. Paraffin-embedded skin specimens were used for conventional histologic staining. A portion of each biopsy specimen was immediately snap-frozen in tissue-freezing medium (Tissuetek) and stored at -80°C .

IMMUNOHISTOCHEMISTRY

A specific mouse monoclonal antibody against amino acids 1158 to 1345 of Flk-1/KDR (Santa Cruz Biotechnology) was used to identify the protein on frozen, acetone-fixed kidney sections that were $5\ \mu\text{m}$ thick. The mouse antibody was detected by means of the avidin-alkaline phosphatase method with affinity-purified rabbit antimouse IgG (Dako) and avidin-alkaline phosphatase complex (1:50 dilution; Dako) in a two-step technique. Alkaline phosphatase was stained with a naphthol substrate and fast red TR chromogen (Dako). Slides were counterstained with hematoxylin. Negative controls were obtained by omitting the primary antibody and using rabbit antimouse serum as the first layer. The protein levels were assessed semiquantitatively (by means of scores ranging from 0 to 100, with higher scores indicating more extensive staining) by two observers who were unaware of the origin of the slides.

IMMUNOFLUORESCENCE AND CONFOCAL-LASER SCANNING MICROSCOPY

Levels of expression of VEGF protein and phosphorylated Akt or p70S6 kinase were evaluated by indirect immunofluorescence and confocal microscopy. The primary antibody used to detect VEGF was a rabbit polyclonal antibody against the 165-, 189-, and 121-amino-acid splice variants of the protein (Santa Cruz Biotechnology).

Phosphorylation of Akt and p70S6 kinase was evaluated with the use of specific antibodies against the phosphorylated, and thus active, form of the enzymes. For each enzyme we performed double-fluorescence immunolabeling to evaluate on the same tissue section the expression of the enzyme and the levels of its activated form. Mouse monoclo-

Table 1. Clinical Characteristics of the Patients.*

Characteristic	Value
Age (yr)	48.7 \pm 7.9
Sex (no. of patients)	
Male	12
Female	3
Panel-reactive antibodies (%)	0
No. of HLA mismatches	2.9 \pm 0.7
Time from transplantation to diagnosis of Kaposi's sarcoma (mo)	
Median	12
Interquartile range	5–75
No. of lesions	
Mean	18
Range	10–45
Serum creatinine (mg/dl)	
Before initiation of sirolimus	1.5 \pm 0.9
6 Mo after initiation of sirolimus	1.4 \pm 0.6
Episodes of acute rejection	
Before onset of Kaposi's sarcoma	0
After initiation of sirolimus	0

* Plus-minus values are means \pm SD. To convert values for creatinine to micromoles per liter, multiply by 88.4.

nal antibody against Akt1 recognizes the sequence 345 to 480 of human Akt1 (Santa Cruz Biotechnology). The antibody against phosphorylated Akt1 was a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated serine at position 473 of human origin (Santa Cruz Biotechnology). A mouse monoclonal antibody was raised against a peptide at the carboxy terminal of rat p70S6 kinase (Santa Cruz Biotechnology). A mouse monoclonal antibody was raised against a peptide containing the phosphorylated serine at position 411 of p70S6 kinase (Santa Cruz Biotechnology). Immobilized primary antibodies were detected with the use of specific fluorescein isothiocyanate-conjugated secondary antibodies (Alexa Fluor 488; 1:200 dilution; Molecular Probes Europe). The sections were mounted in Gel/Mount (Bioptica) and sealed. Negative control sections were prepared by omitting the primary antibody. The immunofluorescence signal was measured by means of a Leica confocal microscope (model TCS SP2) with the use of scores ranging from 0 to 100, with higher scores indicating more extensive staining.

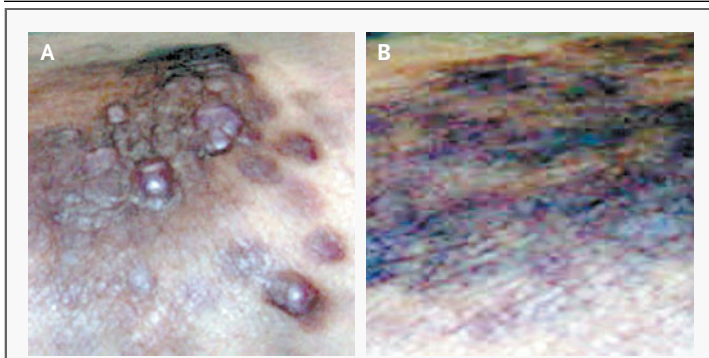


Figure 1. Effect of Sirolimus on Kaposi's Sarcoma Skin Lesions.

Panel A shows the typical appearance of Kaposi's sarcoma in a transplant recipient receiving triple-immunosuppressive-drug therapy (cyclosporine, mycophenolate mofetil, and corticosteroid). Panel B shows the same lesion after one month of sirolimus treatment.

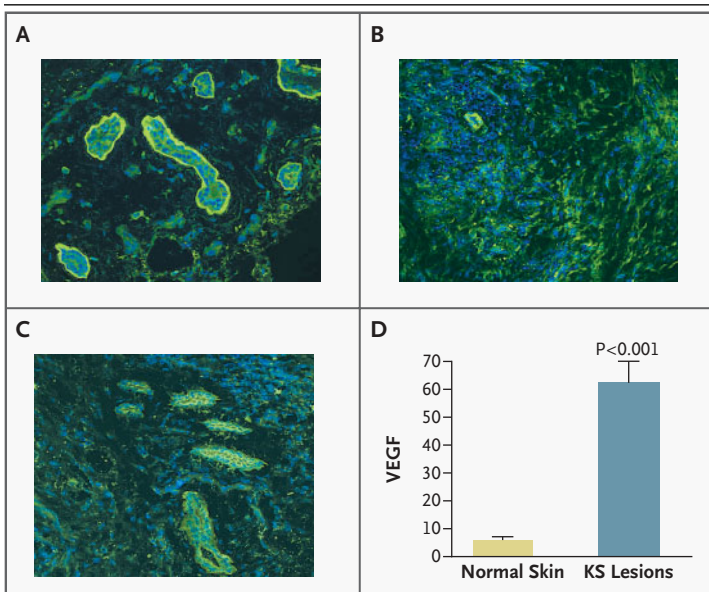


Figure 2. Expression of VEGF Protein.

Panel A shows the expression of VEGF (green) in normal skin, and Panels B and C the expression of VEGF in biopsy specimens of Kaposi's sarcoma (KS) from transplant recipients. Panel D compares the level of expression of VEGF in normal skin and Kaposi's sarcoma lesions. Levels were graded from 0 to 100, with higher scores indicating greater expression. Results are expressed in terms of arbitrary units of immunofluorescence intensity per pixel as mean (\pm SD) values for at least seven patients.

STATISTICAL ANALYSIS

Data are expressed as the means (\pm SD) and compared by analysis of variance. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

PATIENTS

Table 1 shows the principal clinical characteristics of the patients. The mean age was 48.7 ± 7.9 years; 12 of the 15 patients were men. The median time from transplantation to the clinical diagnosis of Kaposi's sarcoma was 12 months (interquartile range, 5 to 75). The number of cutaneous lesions ranged from 10 to 45, with a mean of 18; they were present on the hands, legs, ears, neck, back, and abdomen. Lymphedema was present in 10 patients who had more than 10 lesions on their legs. Our staging protocol for Kaposi's sarcoma includes high-resolution computed tomography of the neck, chest, abdomen, and pelvis; esophagogastroduodenoscopy; and colonoscopy to exclude lymph-node and visceral involvement; no patient was found to have visceral Kaposi's sarcoma.

EFFECT OF SIROLIMUS ON KAPOSI'S SARCOMA

One month after cyclosporine was stopped and sirolimus started, the cutaneous lesions gradually began to disappear in 12 of the 15 patients (Fig. 1). Three months after the initiation of sirolimus, cutaneous Kaposi's sarcoma lesions could not be identified in any patient. To confirm that clinical remission had occurred, in all patients we obtained another skin specimen from the site of a previous Kaposi's sarcoma lesion six months after sirolimus therapy was begun. All such biopsy specimens were negative for Kaposi's sarcoma. During follow-up, target sirolimus blood levels were 6 to 10 ng per milliliter (mean, 7.4) and renal-graft function was stable, with essentially no change in serum creatinine levels (Table 1). Moreover, there were no episodes of acute rejection immediately after cyclosporine was withdrawn or throughout the study (Table 1).

VEGF AND Flk-1/KDR IN SKIN-BIOPSY SPECIMENS

Guba et al.¹⁰ recently demonstrated that the anti-neoplastic effect of sirolimus is primarily mediated by an antiangiogenic effect that is due to the suppression of VEGF. We therefore looked for VEGF and Flk-1/KDR in skin-biopsy specimens of Kaposi's sarcoma lesions obtained after transplantation. VEGF, barely detectable in normal skin (Fig. 2A), was highly expressed by Kaposi's sarcoma cells (Fig. 2B). By contrast, the expression of VEGF was strikingly increased in the normal skin cells around the Kaposi's sarcoma lesions (Fig. 2C). Flk-1/KDR was present in low amounts in normal skin (Fig. 3A),

but the levels were markedly increased within Kaposi's sarcoma cells in biopsy specimens (Fig. 3B, 3C, and 3D).

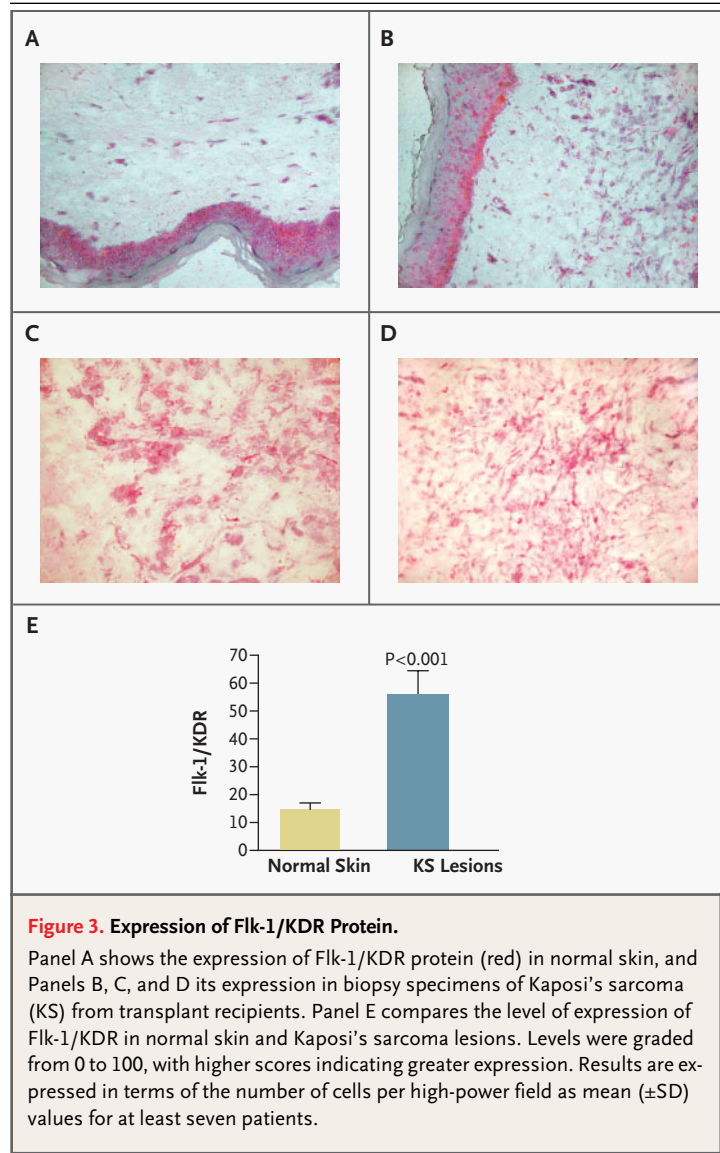
PHOSPHORYLATED Akt-p70S6 KINASE IN SKIN-BIOPSY SPECIMENS

We investigated whether the Akt-p70S6 kinase axis, the signaling pathway disrupted by sirolimus, was activated in transplant recipients with Kaposi's sarcoma, as shown by Sodhi et al.⁹ in patients with AIDS-associated Kaposi's sarcoma. Levels of phosphorylated Akt were low in normal skin (Fig. 4A) but strikingly increased and translocated to the nucleus in Kaposi's sarcoma lesions (Fig. 4B). Levels of phosphorylated p70S6 kinase, present in normal skin only within the basal stratum of the epidermis (Fig. 5A), were increased in Kaposi's sarcoma cells and translocated to the nucleus (Fig. 5B).

DISCUSSION

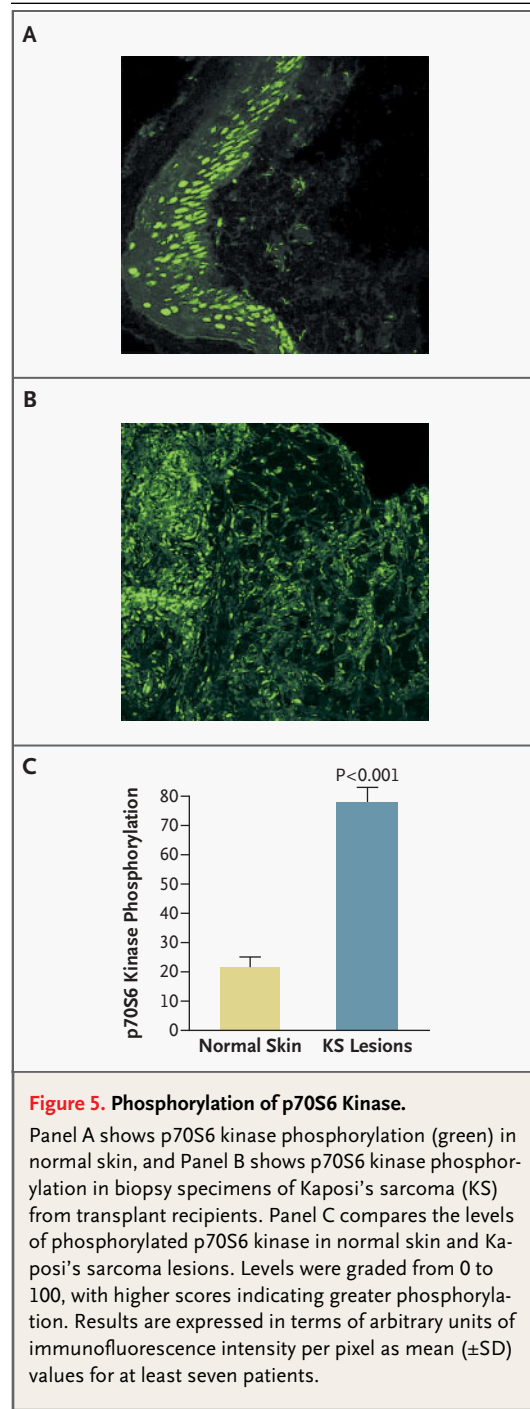
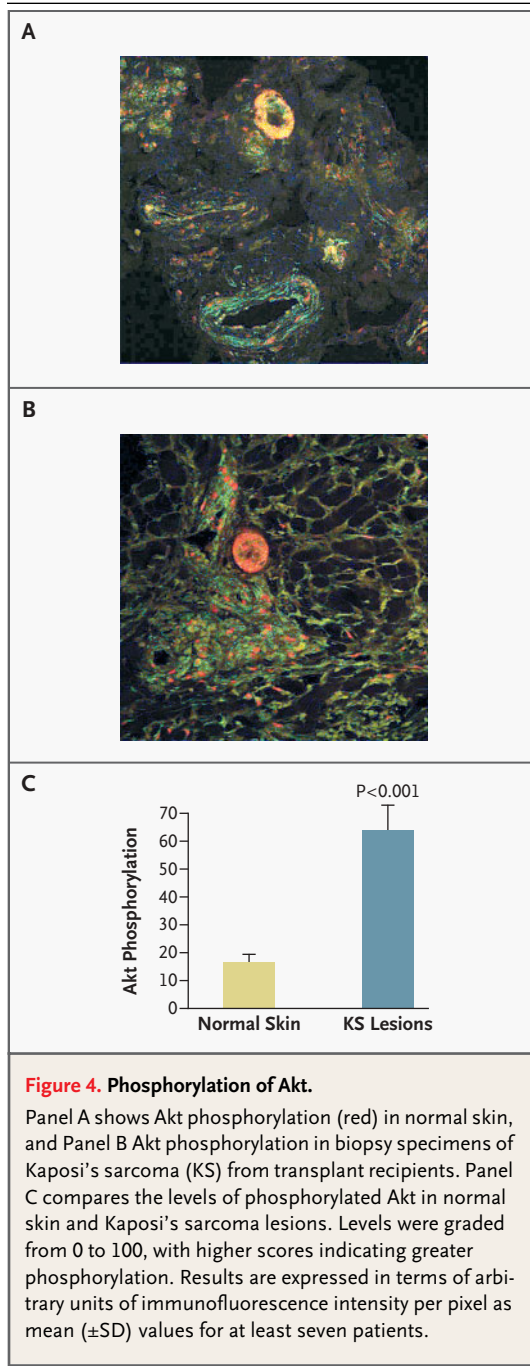
The introduction of highly effective immunosuppressive therapy has reduced the incidence of acute rejection among recipients of kidney transplants but increased the risk of infections and cancer.¹² In this study of 15 renal-transplant recipients, we found that sirolimus, an immunosuppressive agent used in kidney transplantation, inhibits the progression of dermal Kaposi's sarcoma when given at the usual immunosuppressive doses. In all 15 patients, treatment was switched from cyclosporine to the mTOR inhibitor, and all had complete clinical and histologic regression of Kaposi's sarcoma lesions of the skin.

There is experimental evidence that cyclosporine enhances the growth of cancer cells,⁸ inhibits mechanisms of DNA repair,¹³ and increases recurrences of liver tumors in rats.¹⁴ In addition, Shihab et al. demonstrated increased expression of VEGF in a rat model of chronic cyclosporine nephrotoxicity.¹⁵ For these reasons, our observation of increased expression of VEGF within skin cells surrounding Kaposi's sarcoma lesions might be due at least in part to cyclosporine; the cessation of cyclosporine therapy may have reduced the expression of VEGF, an autocrine growth factor for Kaposi's sarcoma cells. This effect of cyclosporine suggests that most of the effects on Kaposi's sarcoma that we observed were due to the cessation of cyclosporine treatment. However, data from the Cincinnati Transplant Tumor Registry indicate that reducing the dose of cyclosporine or stopping treat-



ment leads to regression or disappearance of skin lesions in only 17 percent of patients with Kaposi's sarcoma.¹⁶ We therefore believe that sirolimus also had a role in the regression of Kaposi's sarcoma lesions in our patients.

There is increasing evidence from studies in animals that sirolimus has an antineoplastic action that is independent of its immunosuppressive effect.¹⁷⁻¹⁹ In our patients, the switch from cyclosporine to sirolimus did not provoke acute episodes of rejection and graft function remained stable after treatment with sirolimus was begun. Guba et al.¹⁰ proposed that the antitumor activity of sirolimus was mainly due to its antiangiogenic effect, medi-



ated by a reduction in VEGF and its Flk-1/KDR receptor on endothelial cells. This cellular effect is particularly relevant in the setting of post-transplantation Kaposi's sarcoma, given the pivotal role of the VEGF system in the pathogenesis of this neoplasia.

Several enzymes along the signaling pathway that is inhibited by sirolimus play a role in the devel-

opment and progression of different cancers.²⁰⁻²² For instance, mTOR, the target of sirolimus, is activated by Akt, an antiapoptotic enzyme.²³ Activated (phosphorylated) mTOR activates various signaling mediators, including p70S6 kinase and eIF-4E, two checkpoints of the translation machinery.²³

In skin-biopsy specimens from kidney-transplant recipients with Kaposi's sarcoma, we found increased phosphorylation of both Akt and p70S6 kinase within the Kaposi's sarcoma lesions, which was most likely the result of the activation of VEGF receptors.²⁴

In conclusion, our study suggests that sirolimus inhibits the progression of Kaposi's sarcoma in kidney-transplant recipients while exerting an anti-

rejection effect on organ allografts. This dual role of the drug may prove important in other situations in which transplant recipients are at high risk for tumor recurrence or primary cancer.

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REFERENCES

- Hayward GS. Initiation of angiogenic Kaposi's sarcoma lesions. *Cancer Cell* 2003; 3:1-3.
- Penn I. Cancers in cyclosporine-treated vs azathioprine-treated patients. *Transplant Proc* 1996;28:876-8.
- Doutrelepon JM, De Pauw L, Gruber SA, et al. Renal transplantation exposes patients with previous Kaposi's sarcoma to a high risk of recurrence. *Transplantation* 1996;62:463-6.
- Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994;266:1865-9.
- Masood R, Cai J, Zheng T, Smith DL, Naidu Y, Gill PS. Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. *Proc Natl Acad Sci U S A* 1997;94:979-84.
- Marchiò S, Primo L, Pagano M, et al. Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. *J Biol Chem* 1999;274:27617-22.
- Wiederrecht GJ, Sabers CJ, Brunn GJ, Martin MM, Dumont FJ, Abraham RT. Mechanism of action of sirolimus: new insights into the regulation of G1-phase progression in eukaryotic cells. *Prog Cell Cycle Res* 1995; 1:53-71.
- Hojo M, Morimoto T, Malluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999; 397:530-4.
- Sodhi A, Montaner S, Patel V, et al. Akt plays a central role in sarcomagenesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. *Proc Natl Acad Sci U S A* 2004;101:4821-6.
- Guba M, von Breitenbuch P, Steinbauer M, et al. Sirolimus inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002;8:128-35.
- Luan FL, Ding R, Sharma VK, Chon WJ, Lagman M, Suthanthiran M. Sirolimus is an effective inhibitor of human renal cancer metastasis. *Kidney Int* 2003;63:917-26.
- Morath C, Mueller M, Goldschmidt H, Schwenger V, Opelz G, Zeier M. Malignancy in renal transplantation. *J Am Soc Nephrol* 2004;15:1582-8.
- Herman M, Weinstein T, Korzets A, et al. Effects of cyclosporine A on DNA repair and cancer incidence in kidney transplant recipients. *J Lab Clin Med* 2001;137:14-20.
- Freise CE, Ferrell L, Liu T, Ascher NL, Roberts JP. Effect of systemic cyclosporine on tumor recurrence after liver transplantation in a model of hepatocellular carcinoma. *Transplantation* 1999;67:510-3.
- Shihab FS, Bennett WM, Yi H, Andoh TE. Expression of vascular endothelial growth factor and its receptors Flt-1 and KDR/Flk-1 in chronic cyclosporine nephrotoxicity. *Transplantation* 2001;72:164-8.
- Penn I. Sarcomas in organ allograft recipients. *Transplantation* 1995;60:1485-91.
- Luan FL, Hojo M, Maluccio M, Yamaji K, Suthanthiran M. Sirolimus blocks tumor progression: unlinking immunosuppression from antitumor efficacy. *Transplantation* 2002;73:1565-72.
- Koehl GE, Andrassy J, Guba M, et al. Sirolimus protects allografts from rejection while simultaneously attacking tumors in immunosuppressed mice. *Transplantation* 2004;77:1319-26.
- Campistol JM, Gutierrez-Dalmau A, Torregrosa JV. Conversion to sirolimus: a successful treatment for posttransplantation Kaposi's sarcoma. *Transplantation* 2004;77:760-2.
- Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A* 1999;96:4240-5.
- Ruggero D, Montanaro L, Ma L, et al. The translation factor eIF-4E promotes tumour formation and cooperates with c-myc in lymphomagenesis. *Nat Med* 2004;10:484-6.
- Wendel HG, De Stanchina E, Fridman JS, et al. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 2004;428:332-7.
- Panwalkar A, Verstovsek S, Giles FJ. Mammalian target of sirolimus inhibition as therapy for hematologic malignancies. *Cancer* 2004;100:657-66.
- Gerber HP, McMurtrey A, Kowalski J, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway: requirement for Flk-1/KDR activation. *J Biol Chem* 1998; 273:30336-43.

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