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NEWS IN BRIEF

Coronavirus is the cause of the severe acute respiratory syndrome (SARS) outbreak in Hong Kong and worldwide

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An outbreak of severe atypical pneumonia occurred in Hong Kong in March 2003 (1,2). The condition first appeared among health-care workers and their household members and later spread to the community. The disease apparently originated in Guangdong Province in southern China in November 2002. In late February 2003, the Centers for Disease Control and Prevention (CDC) termed this condition the severe acute respiratory syndrome (SARS) and provided a clinical case definition. Now, SARS has spread worldwide to involve patients in North America, Europe, and other Asian countries.

In less than 2 months from its onset in Hong Kong, Peiris et al. have identified a novel coronavirus as the cause of SARS (3). The RNA virus was isolated from two patients, one from an open lung biopsy sample and the other from a nasopharyngeal aspirate. The virus grew in fetal rhesus kidney cells and appeared as 55- to 90-nm-diameter particles within smooth-walled vesicles in the cytoplasm and at the cell surface. The cell culture extracts revealed pleomorphic enveloped virus particles of around 80 to 90 nm (range 70–130 nm) in diameter, with surface morphology compatible with a coronavirus. Using random RT-PCR assay, a 646-bp fragment of the polymerase gene was found and showed highest homology to viruses of the family of Coronaviridae. By RT-PCR and indirect immunofluorescence assays specific for this virus, 45 of 50 patients with SARS, but no controls, had evidence of infection with the virus. These findings significantly strengthen the tentative etiologic association reported by other investigators from CDC in Atlanta and from Toronto, who have also isolated a novel coronavirus from patients with SARS. Now the genome has been completely sequenced (4,5). Apparently, the virus is not closely related to any known human or animal coronaviruses.

Regarding the pathology of coronavirus pneumonia, preliminary study on the first few fatal cases revealed changes of diffuse alveolar damage in varying phases (1,2). With accumulated experience, a rather distinctive pattern is observed: diffuse alveolar damage accompanied by a desquamative pattern and decorated by scattered multinucleated syncytial pneumocytes (personal communication, *http://www.eelab.com*). The hyaline membranes, intraalveolar exudate of fibrin, and edema are rather patchy, depending on the severity of the disease. There is a prominent and consistent desquamation of pneumocytes, giving a fairly cellular appearance in the alveolar spaces. These pneumocytes often show vacuolated cytoplasm and may show clear nuclei. Multinucleation with a syncytial appearance of scattered pneumocytes is found on careful search. Inflammatory infiltrate is relatively sparse and mainly mononuclear in type and interstitial in location. Organization with globular fibrogranulation tissue, some having a "glomeruloid" appearance, is seen in those alveoli in advanced stage. On electron microscopy, viral particles are present only in cytoplasm and none in the nuclei, which show chromatin clearing. The virus most likely infects the pneumocytes and leads to cytopathic changes as well as immunologic attack, bringing about the pneumonitis. Other organs such as kidney and intestine also harbor the virus and exhibit frequent apoptosis.

The epidemic is just beginning. Characterization of the disease is far from complete, and many questions remain: Does the virus come from animals, and what kind of animals? What is the mode of transmission in those large case clusters? Are there any asymptomatic or mild self-healing cases in the community? Any chronic carriers? What are the best treatment options? Any hope for a vaccine?

1. Tsang KW, HO PL, Ooi GC, et al. A cluster of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* Mar 31, 2003: *http://content.nejm.org/cgi/reprint/NEJMoa030666v2.pdf.*
2. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* Apr 7,2003: *http://content.nejm.org/cgi/reprint/NEJMoa030685v1.pdf.*
3. Peiris JSM, Lai ST, Poon LLM, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003;361: 1319–1325.
4. Rota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* online 30 Apr 2003;10;1126/*science.1085952.*
5. Manta MA, Jones SJM, Astell CR, et al. The genome sequence of the SARS-associated coronavirus. *Science* online 30 Apr 2003; 10;1126/*science.1085953.*

Molecular analysis of surgical margins has no clinical advantage over histologic evaluation

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"Although there may be tumor cells in margins that evade histological detection, their clinical impact appears to be almost negligible." Thus spake Slootweg et al. (1) in their report on the potential value of molecular pathology on margin status in squamous cell carcinomas of the mucosa of the head and neck. Further on in the text, the authors assert their data leave little room for the molecular analysis of surgical margins to improve patient survival by the decrease of mortality due to local recurrence. Proof of this assumption comes from evaluation of 394 patients. In 207 patients, initial treatment was judged complete by conventional histopathologic examination of margins. In 187 patients, there was incomplete excision, as defined as tumor in or close to margin, or mild, moderate, or severe dysplasia or in situ cancer at the margin.

In the group with negative margins, the following information was obtained:

- 16.9% had a second primary head and neck cancer.
- 8.2% had a second tumor (not a metastasis) in the lung.
- 10.6% had recurrent disease in the neck.
- 2.9% had distant metastasis.
- 3.9% had local recurrences at the site of the primary carcinoma.

Of the 41 patients with local recurrences, 39 had invasive carcinoma at the margin and dysplasia occurred in only 2. Of patients in whom histologic examination showed margins to be tumor-free, only 3.9% showed recurrence at the primary site. This difference nicely demonstrates the continued relevance of histologic examination of surgical margins.

Second primary tumors, either in the head and neck or in the lung, are more important in the outcome of patients with squamous cell carcinomas of the head and neck, and these occurred in almost identical percentages in groups with complete and incomplete surgery.

Regarding recurrences in the neck, however, the situation is different. Local recurrences were found in 5 of 76 cases in which a previous elective neck dissection was reported to be tumor-free and that had complete tumor removal at the primary site. While the recurrences might be undetected "micrometastases," it is uncertain if such micrometastases have the same clinical significance as metastases detected by conventional methods (2).

1. Slootweg PJ, Hordijk GJ, Schade Y, et al. Treatment failure and margin status in head and neck cancer. A critical view on the potential value of molecular pathology. *Oral Oncol* 2002;38:500–503.
2. Ferlito A, Devaney KO, Devaney SL, et al. Clinicopathological consultation. Micrometastases: have they an impact on prognosis? *Ann Rhinol Laryngol* 1999;108:1185–1189.

cDNA microarray study of synovial sarcomas

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cDNA microarray study of human tumors offers the unique opportunity to simultaneously analyze the expression of thousands of genes. As such, these studies offer unique perspectives into the molecular mechanisms of carcinogenesis and neoplastic progression and serve as invaluable tools for the identification of novel genes and potential therapeutic targets. Two recent cDNA microarray studies of synovial sarcomas (SS) highlight both the promise and some potential problems with this exciting new technology.

The first study, by Allander et al. (1), examined tissues from 14 SS (10 monophasic [5 SYT-SSX1 and 5 SYT-SSX2], 4 biphasic [3 SYT-SSX1 and 1 SYT-SSX2]), as compared with 4 malignant fibrous histiocytomas (MFH) and 1 fibrosarcoma (FS). Total cellular RNA was extracted from each tumor and RNA from an osteosarcoma cell line was used as a reference RNA to allow normalization of the expression of each clone relative to the reference for each sample. The authors built their own cDNA microarrays, containing 6,548 sequence-verified cDNA's; the criteria for selection of these particular cDNA's were not stated. The authors also constructed a tissue microarray block, containing tissue from 37 genetically confirmed SS. Immunohistochemistry (IHC) for ERBB2, IGFBP2, IGF2, and BCL2 was performed on this tissue microarray block. FISH for ERBB2 was performed using the PathVysion kit. Statistical analysis was performed using previously described methods.

The cDNA microarray experiment revealed 153 genes that distinguished SS from MFH/FS and could reliably distinguish SS from MFH/SS using hierarchical clustering analysis of 50 genes. SS showed notable overexpression of IGFBP2, ERBB2, IGF2, FGFR3, OLFM1, TLE2, CNTNAP1, DRPLA, CRABP1, and PRAME. Monophasic and biphasic SS differed significantly in their expres-

sion of 21 genes, most notably those encoding cytokeratins 5, 7, 8, and 14 and ELF3. No difference was seen in SYT-SSX1 as compared with SYT-SSX2 tumors. By IHC, ERBB2 expression was seen in the epithelial component of all biphasic SS, epithelioid areas were seen in 3 of 9 monophasic SS, and the spindled cells of 7 other monophasic SS. No ERBB2 gene amplification was present by FISH. IGFBP2 expression was also seen in the epithelial component of all biphasic SS and to a lesser degree in the spindled cells in both types of SS. The authors concluded that SS have a distinctive gene expression profile that distinguishes them from other sarcomas, in particular with regard to genes involved in epithelial differentiation (e.g., cytokeratins, ELF3) and neural/neuroectodermal differentiation (e.g., OLFM1, TLE3, CNTNAP1 and DRPLA). ERBB2 was also identified as a potential therapeutic target.

The second study, by Nagayama et al. (2), compared the expression profile of 13 SS (9 monophasic/4 biphasic, 11 SYT-SSX1/3 SYT-SSX2) with that of 34 non-SS (14 MFH, 10 leiomyosarcomas, 3 dedifferentiated liposarcomas, 3 pleomorphic liposarcomas, 4 malignant peripheral nerve sheath tumors [MPNST]). Total cellular RNA was extracted. The authors constructed a "genome-wide" cDNA microarray using 23,040 cDNA's selected from the Unigene database of the National Center for Biotechnology Information. Each microarray slide contained 52 housekeeping genes and the Cy5/Cy3 ratio was adjusted so that the averaged ratio for the panel of housekeeping genes was 1.0. Hierarchical clustering analysis was performed on the entire set of 47 tumors, using a set of 1,204 genes expressed in >90% of cases. The data were also analyzed with regard to particular genes overexpressed by SS and genes overexpressed by subgroups of SS. The expression of a subset of these genes was confirmed with semiquantitative and real-time quantitative RT-PCR. A variety of statistical analyses were performed.

Clustering analysis, using the expression of 1,204 genes, showed SS and MPNST to form a distinct cluster. In contrast, the other sarcomas were scattered and did not form distinct clusters. Two genes, frizzled homolog 10 and an EST, were specifically upregulated in SS, and 24 genes were upregulated in both SS and MPNST, including 2 genes also identified by Allender et al. (1) (CRABP1 and PRAME). The majority of the other overexpressed genes are known to be expressed in neural tissues or involved in neural differentiation, including EphA4, ephrin-B3, Endothelin-3, neurofilament, and neuron-specific protein. The expression of all of these genes was confirmed by RT-PCR. The authors also noted that differences in the expression of 12 genes identified two subgroups of SS, although the exact nature of these subgroups was uncertain, given that significant overlap was seen in the expression profiles of monophasic and biphasic tumors, and in SYT-SSX1 and SYT-SSX2 tumors. There was no mention in this study of other genes noted by Allender et al. to be significantly overexpressed in SS (e.g., ERBB2, IGFBP2). The authors concluded that SS and MPNST were very likely related, that novel subgroups of SS could be identified, and that cDNA expression analysis was a valuable tool for the identification of novel therapeutic targets.

Although both of these studies were carefully performed and provide valuable new insights into SS, certain questions remain. Certainly the findings of these two studies do overlap to a degree, with respect to the overexpression of PRAME and CRABP1, for example. However, one is struck by the fact the majority of the genes found to be most significant by Allender et al. were not identified by Nagayama et al., and vice versa. Although it is certainly conceivable that the much smaller array constructed by Allender et al. might not have included all of the genes included in the larger array of Nagayama et al., it is difficult to imagine that the "genome-wide" array of Nagayama et al. did not include the great majority of the genes included on the smaller array. Conceivably, these differences in expression profiles may be related to technical issues, possibly related to different types of controls, or to differences in statistical analysis. Ultimately, the answers to these questions must await the work of additional investigators. There appears to be an urgent need for the standardization of cDNA microarrays and techniques so that the data of different studies may be easily compared.

1. Allander, et al. Expression profiling of synovial sarcoma by cDNA microarrays: association of ERBB2, IGFBP2, and ELF3 with epithelial differentiation. *Am J Pathol* 2002;161:1587–1595.
2. Nagayama S, et al. Genome-wide analysis of gene expression in synovial sarcomas using a cDNA microarray. *Cancer Research* 2002;62:5859–5866.

Revised staging system for cutaneous melanoma: implications for pathologists and dermatopathologists

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Melanoma is responsible for the majority of all deaths related to neoplasms of the skin. While many factors have been proposed as prognostically significant in melanoma patients, several have shown consistent correlation in multiple studies. A recent multicenter study

by Balch et al. involving over 17,000 melanoma patients analyzed factors that have demonstrated correlation with melanoma prognosis (1). The study demonstrated that tumor thickness and ulceration were the most powerful predictors of survival in the tumor (T) category. In the node (N) category, the number of metastatic lymph nodes and whether the nodes were clinically apparent or clinically occult were independent survival factors. Nonvisceral metastases were associated with a better survival than visceral metastases in the metastasis (M) category. As a result of these studies, the American Joint Committee on Cancer (AJCC) has revised the TMN staging system for cutaneous melanoma (2). The revised melanoma staging system is published in the sixth edition of the *AJCC Cancer Staging Manual* (3). In the revised staging system, there are several important changes that have a direct impact on pathologists and the reporting of melanoma specimens. The major changes in the revised system include the following: revision of Breslow thickness T-stage criteria; removal of Clark's level (except T1 tumors); inclusion of tumor ulceration as a major prognostic factor; use of the number of metastatic lymph nodes rather than nodal size; differentiation of micrometastases from macrometastases; merging of satellite and in-transit metastases into one category; site-specific distant metastases and presence of elevated serum lactic dehydrogenase in M category. The new staging system also incorporates information obtained in sentinel lymph node biopsy. Notable factors often contained in pathology reports but not included in the revised AJCC melanoma staging system include radial growth pattern, number of mitoses, lymphocytic host response, and the presence of regression. The following is a brief description of the significant changes in melanoma staging in the current system and their implications for pathologists and dermatopathologists in the reporting of melanoma specimens.

Tumor (Breslow) thickness

The previous AJCC staging system used tumor thickness thresholds of 0.75, 1.50, and 4.0 mm (4). The revised staging system designates thresholds of 1.0, 2.0, and 4.0 mm. The increases from 0.75 to 1.0 mm and 1.5 to 2.0 mm reflect the finding that no significant prognostic differences have been demonstrated between previous and revised thickness parameters (5). Thus, the new tumor thickness T stages are as follows: T1, $\leq$1.0 mm; T2, 1.01 to 2.0 mm; T3, 2.01 to 4.0 mm; and T4, >4.0 mm.

In addition to melanoma staging, tumor thickness is critical in the decision to perform sentinel node lymphadenectomy. A thickness of $\geq$1.0 mm is used by many surgical oncologists, but thresholds may vary depending on the institution.

Tumor ulceration

One of the most significant additions to the revised staging system is the inclusion of ulceration as a major prognostic factor in melanomas. The presence of ulceration has been shown to correlate with a significantly worse prognosis as compared to nonulcerated tumors (1,6). The presence or absence of overlying epidermal ulceration is defined by microscopic examination. Of the major prognostic factors reported in melanoma, the presence of ulceration is under-reported by pathologists. In the revised staging system, tumors with ulceration are upstaged to reflect the adverse change in prognosis. The negative prognostic impact of melanoma ulceration is seen in stage I, II, and III tumors. Stage IV tumors generally have a poor prognosis with or without ulceration.

Level of invasion (Clark's level)

With the exception of T1 tumors, the level of invasion is not included in the revised AJCC staging system. Studies have shown that the level of invasion is a significant prognostic factor in thin ($\leq$1.00 mm) but not thicker melanomas (1,7). These results in part reflect differences in dermal thickness at different anatomic sites. In the revised system, tumors $\leq$1.00 mm with level II or III invasion and no ulceration are designated as T1a, and tumors $\leq$1.00 mm with level IV or V invasion and/or ulceration are assigned to the T1b category.

Number of positive metastatic lymph nodes

The number of metastatic lymph nodes has demonstrated significant correlation with survival in melanoma patients (1,2,7). Based on these data, lymph node number is used as a primary factor in defining the N category. The threshold values in the revised staging system are 1, 2 or 3, and $\geq$4 lymph nodes involved, corresponding to N1, N2, and N3 respectively. The previous AJCC staging system used the size of nodal metastases, which has not shown independent prognostic significance.

Micrometastases and macrometastases

The use of sentinel lymphadenectomy has substantially improved the detection of clinically occult metastases. The data generated from sentinel lymph node studies have shown prognostic differences between clinically apparent and clinically occult metastases (1,7). In the revised staging system, the metastatic nodal tumor burden is defined in two broad categories, micrometastases and macrometastases. Micrometastasis is defined as metastasis detected by microscopic examination in clinically negative lymph nodes. No distinction is made for

the size of micrometastasis or whether it is apparent in hematoxylin and eosin or immunohistochemical stained sections. Additionally, polymerase chain reaction techniques are not addressed. Macrometastasis is defined as clinically evident nodal metastasis with confirmation on microscopic examination. As with micrometastases, metastatic foci sizes are not included in the revised staging system.

Satellite and in-transit metastases

The previous melanoma staging system included satellite lesions in the T category and in-transit metastases in the N category (4). Although both entities are associated with a poor prognosis, research has shown no significant prognostic difference (7). Therefore, both satellite and in-transit metastases have been merged as a separate N (N2c) category in the revised staging system.

Site of distant metastases

In the previous staging system, all visceral metastases were included together in the same M category (4). Studies have shown that some anatomic sites of distant metastasis have different prognostic implications (1,8). Patients with metastasis to the skin, subcutis, or distant lymph node have a better prognosis than those with visceral metastases. Additionally, patients with lung metastases have shown a better 1-year prognosis than patients with metastases to other visceral sites (8). Survival in patients with any visceral metastasis is poor beyond 1 year. Based on these survival data, the current staging system subdivides the M categories into the following: M1 a, skin, subcutis, or distant nodal metastasis; M1b, lung metastasis; M1c all other visceral and distant metastasis.

In addition to site of distant metastases, elevation of serum lactic dehydrogenase (LDH) is also used in the M category. Elevated LDH has been shown to be an independent predictive factor of decreased survival in melanoma patients with metastases (9). Any patient with distant metastases and an elevated LDH is assigned to the M1c category.

Conclusion

Although the revisions in the melanoma staging system provide crucial data for prognosis, many pathologists and dermatopathologists will also choose to include additional factors, such as mitotic rate, in their reports. As with all staging systems, the incorporation of new technology and new therapies will undoubtedly lead to further refinement of the new staging system and inclusion of additional prognostic factors.

1. Balch CM, Soong S-J, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: Validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001;19:3622–3634.
2. Balch CM, Buzaid AC, Soong S-J, et al. Final version of the American Joint Committee on Cancer Staging system for cutaneous melanoma. *J Clin Oncol* 2001;19:3635–3648.
3. Greene FL, Page DL, Fleming ID, et al. *American Joint Committee Cancer staging manual,* sixth ed. New York: Springer Verlag, 2002.
4. Fleming ID, Cooper JS, Henson DE, et al. *American Joint Committee Cancer staging manual,* fifth ed. Baltimore: Lippincott Williams and Wilkins, 1997.
5. Buttner P, Garbe C, Bertz J, et al. Primary cutaneous melanoma: Optimized cutoff points of tumor thickness and importance of Clark's level for prognostic classification. *Cancer* 1995;75:2499–2506.
6. Balch CM, Wilkerson JA, Murad TM, et al. The prognostic significance of ulceration in cutaneous melanoma. *Cancer* 1980;45:3012–3017.
7. Buzaid AC, Ross MI, Balch CM, et al. Critical analysis of the current American Joint Committee on Cancer staging system for cutaneous melanoma and proposal of a new system. *J Clin Oncol* 1997;15:1039–1051.
8. Barth A, Wanek LA, Morton DL. Prognostic factors in 1,521 melanoma patients with distant metastases. *J Am Coll Surg* 1995;181:193–201.
9. Eton O, Legha SS, Moon TE, et al. Prognostic factors for survival of patients treated systemically for disseminated melanoma. *J Clin Oncol* 1998;16:1103–1111.

Sirolimus (rapamycin) impairs renal function

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The two main causes of long-term renal allograft loss are rejection and drug toxicity. Drug toxicity typically implicates the calcineurin inhibitors, cyclosporine and tacrolimus (FK506), and manifests as multiple histologic patterns, including isometric tubular vacuolization, hyaline arteriolopathy, stripe-like interstitial fibrosis, and thrombotic microangiopathy.

Standard triple immunosuppressive regimens administered to renal transplant recipients include a calcineurin inhibitor, low-dose prednisone and either azathioprine or mycophenolate mofetil. More recently, sirolimus (rapamycin/Rapamune, Wyeth Pharmaceuticals, Madison, NJ) has been increasingly used instead of azathioprine or mycophenolate mofetil; all three of these agents were previously thought to have minimal nephrotoxicity.

Sirolimus is a macrolide lactone isolated from *Streptomyces hygroscopicus.* Its use in renal transplantation has increased over the past decade because of its apparent lack of renal toxicity and its effectiveness in reducing the incidence of acute rejection in multiple clinical trials (1–3). Side effects of sirolimus include thrombocytopenia, leukopenia, hypercholesterolemia, hypertriglyceridemia, hypokalemia, elevated liver enzymes, and an in-

creased incidence of herpes simplex and pneumonia (1–4). A major benefit of sirolimus is its potential for decreasing the dose of or avoiding to use of calcineurin inhibitors. Two trials have documented comparable renal function and rejection rates when calcineurin inhibitors have been withdrawn or avoided in regimens containing sirolimus (4,5). Thus, sirolimus-based immunosuppressive regimens have the potential to avoid the chronic nephrotoxicity associated with calcineurin inhibitor use.

The mechanism of action of sirolimus differs from other available immunosuppressive agents. Cyclosporine, tacrolimus, and sirolimus all produce their immunosuppressive effect by binding to cytoplasmic proteins ("immunophilins") that modify immune function. Cyclosporine binds cyclophilin and tacrolimus binds FK binding protein 12 (FKBP12); these complexes in turn inhibit calcineurin, a calcium-dependent phosphatase required for interleukin-2 production and progression of T cells from the G_0 to G_1 phase of the cell cycle. Sirolimus also binds FKBP12, but the complex does not inhibit calcineurin activity. In contrast, the sirolimus–FKBP12 complex inhibits a cell cycle regulatory protein referred to as mammalian target of rapamycin (mTOR) (6). Inhibition of mTOR blocks interleukin production and, progression of T cells from the G_1 to S phase of the cell cycle.

Recently, the belief that sirolimus lacks the ability to impair renal function has been questioned (7–9). In an animal model of ischemic acute tubular necrosis, sirolimus delayed recovery of renal function (7). In contrast, sirolimus had no effect on renal function in sham-operated animals. Delayed recovery of renal function in sirolimus-treated rats was associated with increased tubular cell apoptosis and reduced cellular proliferation. Based on additional studies in mouse cultured proximal tubular cells, the effect of sirolimus may be mediated by inhibition of 70-kDa S6 protein kinase (7). These experiments suggest that while sirolimus is not a direct nephrotoxin, in the setting of ischemic-reperfusion injury sirolimus impairs recovery of renal function via its effects on cell proliferation and apoptosis. From these experiments, one might predict that sirolimus would be associated with delayed graft function (DGF).

An association between sirolimus and DGF has been confirmed in a recent, important, retrospective case-controlled study on patients with delayed graft function who had received a renal transplant at the University of Washington Medical Center (8). Patients were given one of four immunosuppressive regimens in a non-randomized fashion. Two of the regimens, given to 88 of the 144 patients, included sirolimus co-administered with tacrolimus. The findings were striking: the prevalence of DGF was 25% in patients treated with sirolimus versus 8.9% in those who did not receive sirolimus ($P = 0.02$). Furthermore, the dose of sirolimus correlated with the risk of DGF.

Renal biopsies from the 22 patients treated with sirolimus who developed DGF revealed widespread tubular injury, typical of what may be seen in the setting of acute tubular necrosis (8). In many cases, the biopsies were repeated at a later date. Twelve of the 22 renal biopsies, all of which were performed at least 3 weeks post-transplantation, when the patients were also receiving tacrolimus, exhibited a previously undescribed pattern of cast nephropathy that closely resembled the changes seen in myeloma cast nephropathy (MCN). Intratubular, atypical, angulated, eosinophilic, fractured casts that were focally associated with multinucleated giant cells of the monocyte/macrophage lineage accompanied the tubular injury. Many of the casts contained eosinophilic bodies with prominent borders; these structures were composed of degenerated tubular epithelial cells, as noted by electron microscopy and immunohistochemical staining for cytokeratin. The authors suggested that unlike in MCN, where light chains are the central component of the tubular casts, the casts associated with sirolimus and tacrolimus are centered around degenerated tubular epithelium. In three patients with DGF and cast nephropathy, the renal insufficiency resolved following discontinuation of tacrolimus and sirolimus, treatment for 2 weeks with thymoglobulin, and re-introduction of tacrolimus with mycophenolate mofetil and prednisone. A second recent study on cadaveric renal transplant recipients also has linked sirolimus to DGF (9).

The significance of these findings remains to be fully elucidated. There is great need for less toxic immunosuppressive regimens that do not diminish renal function. The initial claims that sirolimus does not impair renal function appear flawed, and the full spectrum of sirolimus's detrimental effects on renal function are incompletely understood. Further research should address interactions between sirolimus and other immunosuppressive agents with respect to efficacy and nephrotoxicity, the spectrum of sirolimus nephrotoxicity in the absence of calcineurin inhibitors, and the value of administering sirolimus during the early post-transplant period when DGF occurs.

1. Kahan BD, Podbielski J, Napoli KL, et al. Immunosuppression effects and safety of a sirolimus/cyclosporine combination regimen for renal transplantation. *Transplantation* 1999;66:1040–1046.
2. The RAPAMUNE Global Study Group. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 2001;27:271–280.

3. Ponticelli C, MacDonald AS, Rajagopalan P, et al. Phase II trial of rapamycin versus placebo in primary renal allograft recipients. *Transplant Proc* 2001;33:2271–2272.
4. Groth CG, et al. Sirolimus (Rapamycin)–based therapy in human renal transplantation. *Transplantation* 1999;67:1036–1042.
5. Gonwa TA, Hricik DE, Brinker K, et al. Improved renal function in sirolimus-treated renal transplant recipients after early cyclosporine elimination. *Transplantation* 2002;74:1560–1567.
6. Schmelzle T, Hall MN. TOR, a central controller of cell growth. *Cell* 2000;103:253–262.
7. Lieberthal W, Furho R, Andry CC, et al. Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol* 2001;281: F693–F706.
8. Smith KD, Wrenshall LE, Nicosia RF, et al. Delayed graft function and cast nephropathy Issociated with tacrolimus plus rapamycin use. *J Am Soc Nephrol* 2003;14:1037–1045.
9. McTaggart RA, Gottlieb D, Brooks J, et al. Sirolimus prolongs recovery from delayed graft function after cadaveric renal transplantation. *Am J Transplant* 2003;3:416–423.