

ORIGINAL ARTICLE

# Antibodies against MICA Antigens and Kidney-Transplant Rejection

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## ABSTRACT

### BACKGROUND

Good HLA-A, HLA-B, and HLA-DR matches do not guarantee rejection-free renal transplantation. Some kidney transplants fail despite such matches, suggesting that other antigens might be targets for rejection. Major-histocompatibility-complex (MHC) class I-related chain A (MICA) antigens are polymorphic and can elicit antibody production. We sought to determine whether an immune response to MICA antigens might play a role in the failure of kidney allografts.

### METHODS

Pretransplantation serum samples from 1910 recipients of kidney transplants from deceased donors were tested for anti-MICA antibodies with an assay in which single MICA antigens were attached to polystyrene microspheres.

### RESULTS

Antibodies against MICA alleles were detected in 217 of the 1910 patients (11.4%). The presence of MICA antibodies was associated with renal-allograft rejection. The mean ( $\pm$ SE) 1-year graft-survival rate was  $88.3\pm 2.2\%$  among recipients with anti-MICA antibodies as compared with  $93.0\pm 0.6\%$  among recipients without anti-MICA antibodies ( $P=0.01$ ). Among recipients of first kidney transplants, the survival rate was even lower among MICA antibody-positive patients ( $87.8\pm 2.4\%$ ) than among MICA antibody-negative recipients ( $93.5\pm 0.6\%$ ,  $P=0.005$ ). In addition, the association of MICA sensitization with reduced graft survival was more evident in kidney-transplant recipients with good HLA matching: among 326 recipients who received well-matched kidneys (0 or 1 HLA-A plus HLA-B plus HLA-DR mismatch), sensitization against MICA was associated with poorer allograft survival ( $83.2\pm 5.8\%$  among those with anti-MICA antibodies vs.  $95.1\pm 1.3\%$  among those without such antibodies,  $P=0.002$ ).

### CONCLUSIONS

Presensitization of kidney-transplant recipients against MICA antigens is associated with an increased frequency of graft loss and might contribute to allograft loss among recipients who are well matched for HLA.

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**M**AJOR-HISTOCOMPATIBILITY-COMPLEX (MHC) class I-related chain A (MICA) antigens are surface glycoproteins<sup>1</sup> with functions related to innate immunity.<sup>2-4</sup> MICA antigens are expressed on endothelial cells, dendritic cells, fibroblasts, epithelial cells, and many tumors, but not on peripheral-blood lymphocytes. As with the HLA antigens, which MICA antigens resemble only remotely in terms of structure, exposure to allogeneic MICA during transplantation can elicit antibody formation.<sup>5</sup> It is well known that antibodies against HLA antigens, especially those recognizing donor antigens, can severely damage kidney allografts.<sup>6</sup> Transplantation performed in the presence of a positive donor cross-match may result in hyperacute vascular rejection.<sup>7,8</sup> Antibodies against HLA antigens have also been associated with both acute vascular rejection<sup>9,10</sup> and chronic rejection.<sup>11</sup> Since MICA antigens are not expressed on lymphocytes,<sup>12</sup> the cells commonly used for cross-matching, antibodies directed against MICA are not detected with the methods generally used. However, polymorphic MICA antigens are expressed on endothelial cells<sup>12</sup> and have been found to be cytotoxic in the presence of serum complement,<sup>13</sup> so it is likely that such antibodies are harmful to vascularized organ allografts.

Preliminary studies with small numbers of patients indicated that MICA antibodies detected after transplantation might be associated with impaired survival of kidney allografts,<sup>13-17</sup> and an analysis of eluates from kidneys undergoing immunologic rejection has suggested that MICA antibodies may be involved in the pathogenesis of kidney-allograft rejection.<sup>18</sup> Using the resources of the Collaborative Transplant Study, we analyzed serum samples obtained before transplantation from 1910 kidney-transplant recipients. We tested the serum samples for antibodies against MICA antigens and correlated the results with the clinical outcome.

## METHODS

### SPECIMEN COLLECTION

Pretransplantation serum samples from 1910 kidney recipients who underwent transplantation with organs from deceased donors between 1990 and 2004 were provided by 20 centers in 13 countries. The centers included in this analysis pro-

vided written assurance of compliance with local ethical and consent guidelines and of patients' written consent for the use of data for scientific analysis. The study was approved by the ethics committee of the University of Heidelberg.

### LABORATORY TESTS

IgG anti-HLA class I reactivity in the serum samples was tested with the use of Quickscreen enzyme-linked immunosorbent assay (ELISA) kits and for IgG anti-HLA class II reactivity with the use of B-Screen ELISA kits (GTI Diagnostics). HLA typing and panel-reactive HLA antibodies were determined by the tissue-typing laboratories at the participating centers.

Tests for IgG antibodies against MICA antigens were performed with the use of soluble MICA antigens produced in insect cells coupled to polystyrene microbeads (Luminex) in our laboratory in Dallas. These antigens were based on our previous complementary DNA constructs of MICA\*001, MICA\*002, MICA\*004, MICA\*008, and MICA\*009.<sup>18</sup> Fragments encoding the signal peptide and the extracellular domains of these proteins, including a recognition sequence for enzymatic biotinylation and a six-histidine coding sequence with a stop codon, were cloned into pEnter-3C vector (Invitrogen) and selected after sequencing. A baculovirus system was used to produce soluble proteins in High Five insect cells (Invitrogen). These recombinant proteins were affinity purified by nickel-affinity agarose and coupled in duplicate to carboxylated polystyrene microspheres and, after biotinylation, to LumAvidin microspheres (Luminex).

For the assay, serum samples or control samples were incubated with both sets of beads and reacted with phycoerythrin-conjugated goat anti-human IgG (One Lambda). Fluorescence was read with a Luminex 100 flow cytometer (Luminex). A threshold was determined for each bead from the mean value for the relative amount of binding plus 3 SD in serum samples from 21 healthy persons. According to the relative amount of binding and the threshold for each bead, a score of 1 was negative, 2 was doubtful, 4 was weakly positive, 6 was positive, and 8 was strongly positive. For the analysis of graft outcome, scores of 1 and 2 were considered to be negative and scores of 4 to 8 were considered to be positive. Reactions were considered to be positive when the carboxyl-

ated-coupled and the avidin-coupled beads with a given antigen were both positive.

#### STATISTICAL ANALYSIS

Allograft function was analyzed 3, 6, and 12 months after transplantation. No patients were excluded for any reason. Twenty-three patients (1.2%) were lost to follow-up within 3 to 6 months after transplantation, and 34 (1.8%) were lost to follow-up between 6 and 12 months after transplantation. Actuarial graft-survival rates were computed according to the Kaplan–Meier method<sup>19</sup> and expressed as mean ( $\pm$ SE) percentages. Graft survival was compared for patients with and those without MICA antibodies by means of log-rank analysis. In addition, multifactorial Cox regression analysis was performed<sup>20</sup> to consider the effect of potential confounders. In this second analysis, we examined the following covariables: the year of transplantation; the number of grafts; the ages of the donor and recipient; and the patient's sex and race, original disease, geographic region (continent), number of HLA-A plus HLA-B plus HLA-DR mismatches, percentage of panel-reactive antibodies, HLA class I and class II antibodies, cold-ischemia time, type of immunosuppression, and number of pretransplantation blood transfusions. Results are given as adjusted hazard ratios. The software packages SPSS (version 14.0) and SAS (version 8.2) were used for statistical analysis.

#### RESULTS

Table 1 lists the characteristics of the patients. Only three variables were significantly different among the two groups of patients. There were slightly more MICA antibody–positive serum samples from patients who underwent transplantation between the years 2000 and 2004 than among those who underwent transplantation in earlier years. Because serum samples were selected for this study on the basis of available sample volume, aliquots of specimens collected in earlier years were, in some cases, depleted or reduced to an insufficient quantity for the MICA antibody assay; hence, these earlier sample groups were relatively small. Also, the duration of cold ischemia was slightly longer in the MICA antibody–negative group (Table 1), and a few more patients in the MICA antibody–positive group received mycophe-

nolic acid as part of their immunosuppressive regimens.

A total of 217 of the 1910 transplant recipients whose serum was tested for anti-MICA antibodies before transplantation (11.4%) were found to have antibodies against one or more MICA alleles. The presence of MICA antibodies was associated with an increased rate of kidney-allograft rejection (Fig. 1). Patients with anti-MICA antibodies had a 1-year graft-survival rate of  $88.3\pm 2.2\%$  as compared with  $93.0\pm 0.6\%$  among patients in the MICA antibody–negative group ( $P=0.01$ ). The difference between the groups was maintained at 5 years after transplantation. When only recipients of first transplants were considered, the graft-survival rate was  $87.8\pm 2.4\%$  among 183 MICA antibody–positive patients as compared with  $93.5\pm 0.6\%$  among the 1473 recipients who were MICA antibody–negative ( $P=0.005$ ). In addition, the association of MICA sensitization with reduced graft survival was more evident in kidney-transplant recipients with good HLA matching. Among 326 recipients who received kidneys that were well matched (0 or 1 HLA-A plus HLA-B plus HLA-DR mismatch), sensitization against MICA was associated with reduced allograft survival ( $83.2\pm 5.8\%$  among those with MICA antibodies vs.  $95.1\pm 1.3\%$  among those without such antibodies; hazard ratio for allograft loss, 4.97;  $P=0.002$ ). In contrast, MICA antibodies were associated with only a small reduction in allograft survival, which did not reach statistical significance, for recipients with less well-matched kidneys (2 to 4 HLA-A plus HLA-B plus HLA-DR mismatches; hazard ratio for allograft loss, 1.29;  $P=0.36$ ) or poorly matched kidneys (5 to 6 HLA-A plus HLA-B plus HLA-DR mismatches; hazard ratio for allograft loss, 1.63;  $P=0.50$ ).

It appears that the correlation between the presence of anti-MICA antibodies and reduced kidney-allograft survival was not influenced by the simultaneous presence of antibodies against HLA, since this was a rare occurrence. Among 1910 patients tested for HLA class I antibodies, only 37 (1.9%) had both MICA antibodies and antibodies against HLA class I antigens, and only 35 (1.8%) had both anti-MICA and anti-HLA class II antibodies. Moreover, the presence of antibodies against MICA was highly correlated with a poor outcome among recipients who were not sensitized against HLA (Fig. 2). Thus, among 1626

<b>Table 1. Characteristics of the Study Population.*</b>			
<b>Characteristic</b>	<b>MICA-Negative Group (N=1693)†</b>	<b>MICA-Positive Group (N=217)</b>	<b>P Value</b>
Geographic origin — no. (%)			0.17
Europe	1220 (72)	166 (76)	
North America	473 (28)	51 (24)	
Transplantation year — no. (%)			0.037
1990–1994	359 (21)	30 (14)	
1995–1999	264 (16)	35 (16)	
2000–2004	1070 (63)	152 (70)	
Transplant number — no. (%)			0.27
First transplant	1473 (87)	183 (84)	
Subsequent transplant	220 (13)	34 (16)	
Recipient sex — no. (%)			0.07
Male	1031 (61)	146 (67)	
Female	662 (39)	71 (33)	
Recipient race — no./total no. (%)†			0.11
White	1483/1539 (96)	189/201 (94)	
Nonwhite	56/1539 (4)	12/217 (6)	
Latest panel-reactive HLA-antibody value — no. (%)			0.12
0%	1449 (86)	177 (82)	
>0%	244 (14)	40 (18)	
Recipient age — yr	47.3±13.8	47.0±14.5	0.82
Donor age — yr	42.6±16.8	44.0±17.3	0.18
Cold-ischemia time — hr	19.7±7.6	18.1±6.7	0.007
HLA-A+B+DR mismatches — no./total no. (%)			0.07
0–1	284/1687 (17)	42/217 (19)	
2–4	1240/1687 (74)	144/214 (67)	
5–6	163/1687 (10)	30/217 (14)	
Calcineurin inhibitor use — no./total no. (%)			0.12
Cyclosporine	1068/1685 (63)	150/214 (70)	
Tacrolimus	547/1685 (32)	55/211 (26)	
No calcineurin inhibitors	70/1685 (4)	10/217 (5)	
Antiproliferative use — no. (%)			0.006
Azathioprine	426 (25)	56 (26)	
Mycophenolic acid	1027 (61)	146 (68)	
Neither	232 (14)	13 (6)	
Corticosteroid use — no. (%)			0.97
Corticosteroids	1662 (99)	212 (98)	
No corticosteroids	23 (1)	3 (1)	
Induction status			0.59
Induction — no. (%)	665 (39)	89 (41)	
No induction — no./total no. (%)	1020/1672 (61)	126/213 (59)	

\* Plus-minus values are means ±SD. Percentages may not sum to 100 owing to rounding. MICA denotes major-histo-compatibility-complex class I-related chain A.

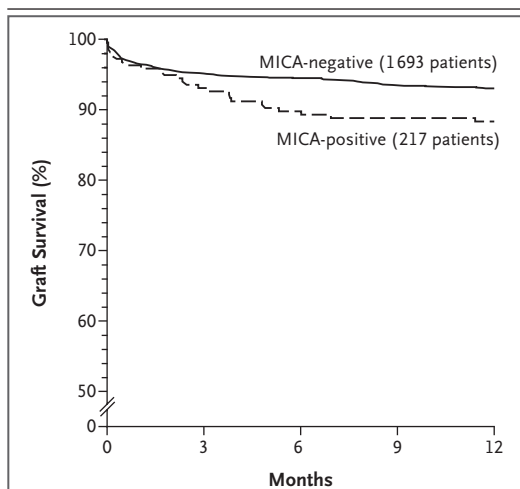
† Race was reported by the patients.

patients with 0% panel-reactive HLA antibodies, there was a higher rate of rejection-related allograft loss among 177 patients with antibodies against MICA. The allograft-survival rate in this group was  $87.4 \pm 2.5\%$ , as compared with  $93.4 \pm 0.7\%$  among recipients with 0% panel-reactive HLA antibodies and no antibodies against MICA ( $P=0.004$ ).

With the use of multivariate analysis, we examined the relationship between the presence of antibodies against MICA among different subpopulations of patients (Table 2). There was a significant negative association between MICA antibodies and allograft survival among first kidney-transplant recipients (hazard ratio for allograft loss, 1.86; 95% confidence interval [CI], 1.17 to 2.97;  $P=0.009$ ), patients who received a transplant with 0 or 1 mismatches for HLA-A, HLA-B, or HLA-DR antigens (hazard ratio for allograft loss, 5.19; 95% CI, 1.94 to 13.88;  $P=0.001$ ), and patients with no panel-reactive HLA antibodies (hazard ratio for allograft loss, 1.85; 95% CI, 1.23 to 2.78;  $P=0.003$ ). There were no significant effects on the outcome for any covariable with antibodies against MICA.

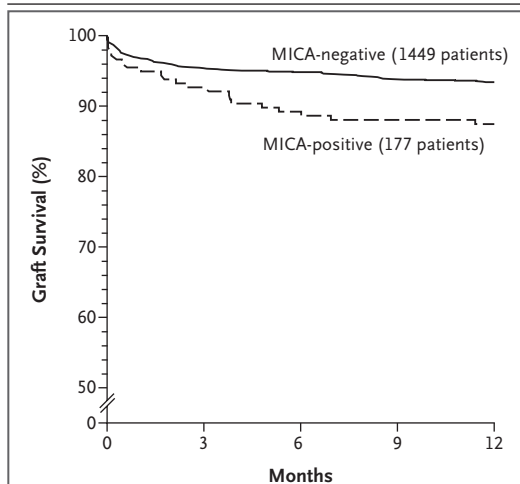
We attempted to investigate the source of the immunization that leads to the production of antibodies against MICA in recipients awaiting kidney transplants. We examined the number of transfusions received by patients with and those without antibodies against MICA and compared these results with the number of transfusions received by recipients with antibodies against HLA class I and class II antigens (Table 3). Among 142 patients with antibodies against HLA class I as determined by means of ELISA, 48.6% had received six or more transfusions. In contrast, among 1100 recipients without HLA class I antibodies, only 16.9% had received six or more transfusions ( $P<0.001$ ). Similarly, the proportion of patients with HLA class II antibodies who had received six or more transfusions was increased by a factor of more than 2 as compared with patients without such antibodies ( $P<0.001$ ) (Table 3). In contrast, the frequency of transfusions did not differ significantly between recipients with and those without antibodies against MICA ( $P=0.15$ ) (Table 3). This result suggests that it is likely that antibodies against MICA were not produced in response to immunization by transfusions.

We did observe, however, that patients sensitized against HLA antigens were somewhat more



**Figure 1. Effect of Antibodies against MICA Antigens in Pretransplantation Serum on the Survival of Kidney Transplants from Deceased Donors.**

Graft survival is shown for all 1910 patients in the study. The rate of graft survival was lower among the 217 recipients who had antibodies against major-histocompatibility-complex class I-related chain A (MICA) before transplantation ( $P=0.01$ ).



**Figure 2. Effect of Antibodies against MICA Antigens in Pretransplantation Serum on Kidney-Graft Survival in Relation to Panel-Reactive HLA Antibodies.**

Graft survival is shown for the 1626 patients who were not sensitized against HLA antigens before transplantation (0% panel-reactive HLA antibody value). The rate of graft survival was lower among the 177 recipients with antibodies against major-histocompatibility-complex class I-related chain A (MICA) ( $P=0.004$ ).

likely to have antibodies against MICA as well. Thus, MICA antibodies were found in 37 of 220 patients with HLA class I antibodies as determined by means of ELISA (16.8%), as compared

**Table 2. Influence of Antibodies against MICA in Subgroups of Patients.\***

Group	No. of Patients	Hazard Ratio (95% CI)†	P Value
All patients	1910	1.63 (1.05–2.52)	0.028
Transplant number			
First transplant	1656	1.86 (1.17–2.97)	0.009
Subsequent transplant	254	0.77 (0.22–2.70)	0.68
HLA-A+B+DR mismatches			
0–1	326	5.19 (1.94–13.88)	0.001
2–4	1384	1.35 (0.78–2.34)	0.29
5–6	193	1.79 (0.41–7.83)	0.44
Latest panel-reactive HLA antibody value			
0%	1626	1.85 (1.23–2.78)	0.003
>0%	284	0.88 (0.26–3.01)	0.84

\* MICA denotes major-histocompatibility-complex class I–related chain A.

† Hazard ratios are for transplant rejection in patients who had antibodies against MICA as compared with patients who did not.

with 180 of 1684 recipients (10.7%) without HLA class I antibodies ( $P=0.007$ ). Similarly, MICA antibodies were present in 35 of 204 patients with HLA class II antibodies (17.2%) as determined by means of ELISA, as compared with 182 of 1692 (10.8%) of those without HLA class II antibodies ( $P=0.007$ ).

## DISCUSSION

Our study provides evidence that sensitization against MICA antigens before transplantation was associated with decreased renal-allograft survival. The graft loss associated with anti-MICA antibodies appears to occur early in the post-transplantation period, a typical feature of rejection mediated by preformed antibodies. We have previously reported that some patients awaiting kidney transplantation had antibodies against certain MICA alleles.<sup>5,18</sup> Among patients in whom an allograft was rejected, the frequency of antibodies against MICA was higher,<sup>16</sup> in keeping with increased expression of MICA and MHC class I–related chain B (MICB) in organs undergoing rejection.<sup>21,22</sup> Our findings suggest that patients with antibodies against MICA before transplantation did not receive more transfusions than patients without such antibodies. These findings are in sharp contrast to the known effect of transfusions in the production of antibodies against class

I and class II HLA antigens; this effect was clearly shown in our analysis of serum samples from the same group of patients. Thus, transfusions were unlikely to have caused the appearance of antibodies against MICA. We speculate that cross-reactivity with substances from the environment may play a role in priming the immune system, facilitating MICA antibody production.

The association of MICA antibodies with allograft rejection was most clearly observed among recipients who received grafts that were well matched in terms of HLA-A plus HLA-B plus HLA-DR. When anti-MICA antibodies were detected before transplantation, the loss of grafts within the first 3 months after transplantation was more common, but differences between the groups were maintained at the end of the first year after transplantation and beyond. The association of antibodies against MICA antigens and allograft rejection was strong in the group of recipients without panel-reactive HLA antibodies, suggesting that the immune response to MICA antigens might play a role in rejection in the absence of previous sensitization against HLA. This association with antibodies against MICA was significant in groups of patients commonly considered to be at low risk for allograft rejection: recipients of first transplants, patients who received grafts from well–HLA-matched donors, and recipients not previously sensitized against HLA. In these patients, kidney transplants were lost significantly more often when antibodies against MICA were present before the transplantation. Thus, we hypothesize that the failure of some well-matched, low-risk kidney transplants might be explained by a heretofore undetected immune response against MICA antigens.

Our study has certain limitations. Typing of the donors for MICA antigens could not be performed because donor DNA was not available; thus, formal proof of donor specificity could not be obtained. Also, the mechanism by which antibodies against MICA antigens develop in recipients before transplantation remains unknown.

Because of the strong effect of the HLA antigens in transplant rejection, the role of minor histocompatibility antigens in kidney-transplant rejection has not received much attention. However, kidneys from HLA-identical siblings do fail at a rate in keeping with the lower immunogenicity of the minor histocompatibility loci. Recently one of us reported that transplants from HLA-

identical siblings fail more frequently among recipients who have higher levels of panel-reactive antibodies than among those with lower levels,<sup>23</sup> suggesting that an immune response against antigens other than HLA might play a determining role in kidney-graft failure. Since the MICA locus is within the MHC, recipients and donors who are HLA-identical by descent would also invariably be matched for MICA. Therefore, the observed difference in graft survival among recipients of transplants from HLA-identical siblings cannot be attributed to MICA. In unrelated transplants well matched for HLA-A, HLA-B, and HLA-DR, however, mismatching for MICA may occur. The association between antibodies against MICA and allograft rejection was most evident in this well-matched and low-risk group in our study.

Polymorphisms distinct from those of the HLA system may also affect the outcome of kidney allografts. Antibodies against endothelial cells were observed among many recipients in whom kidney allografts were rejected.<sup>24-26</sup> Other polymorphisms possibly associated with kidney-allograft failure are those involving vimentin,<sup>27</sup> platelet-specific antigens,<sup>28</sup> genes that encode various cytokines,<sup>29</sup> chemokines and their receptors,<sup>30</sup> and molecules of the renin-angiotensin system.<sup>31</sup>

Our earlier studies indicated that antibodies against MICA alleles are produced after transplantation,<sup>5</sup> and several subsequent studies have shown similar findings.<sup>13-17</sup> The possibility that MICA antigens might be targets during organ-transplant rejection is strengthened by the finding that these antigens are expressed on endothelial cells,<sup>12</sup> that MICA, as well as MICB, can be detected in kidneys undergoing rejection,<sup>21,22</sup> and that anti-MICA antibodies can kill target cells in the presence of serum complement.<sup>13</sup> T cells can recognize and proliferate in response to MICA antigens.<sup>32</sup> Other studies have shown that the frequency of antibodies against MICA increases after transplantation<sup>5,18</sup> and that the frequency is higher among recipients who undergo kidney-allograft rejection as compared with recipients who do not undergo rejection.<sup>17</sup> Additional evidence suggesting that MICA antigens might be directly involved in kidney-transplant rejection came from a study in which, in collaboration with Grosse-Wilde and coworkers, we analyzed

**Table 3. Effect of the Number of Pretransplantation Transfusions on Antibodies against HLA Class I, HLA Class II, or MICA.\***

Antibodies	Transfusions		P Value
	0-5 (N=987) no. (%)	≥6 (N=255) no. (%)	
HLA class I			<0.001
Negative	914 (92.6)	186 (72.9)	
Positive	73 (7.4)	69 (27.1)	
HLA class II			<0.001
Negative	910 (92.2)	202 (79.2)	
Positive	77 (7.8)	53 (20.8)	
MICA			0.15
Negative	866 (87.7)	232 (91.0)	
Positive	121 (12.3)	23 (9.0)	

\* MICA denotes major-histocompatibility-complex class I-related chain A.

59 acid eluates from kidneys determined to have been lost as a result of immunologic rejection.<sup>18</sup> Eleven of the eluates contained anti-MICA antibodies, and five eluates contained antibodies against MICA but not against HLA.

Our current study shows increased rates of allograft failure among patients with antibodies against MICA before transplantation. Anti-MICA antibodies can be readily detected in serum from patients on the transplant waiting list, and recipients at risk for allograft failure might thus be identified.

Although we favor the hypothesis that MICA antibodies are causally involved in allograft rejection, we have not formally proved that the antibodies that correlate with decreased graft survival are specific for the MICA antigens of the donor. If that turns out to be the case, it will be important to develop strategies to reduce or eliminate the effect of MICA antibodies on kidney-graft outcome.

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