Enhanced Kidney Allocation to Highly Sensitized Patients by the Acceptable Mismatch Program

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Even in this era of efficient immunosuppression, a positive serological crossmatch caused by complement fixing antibodies directed against the human leukocyte antigen (HLA) mismatches of the organ donor is a contraindication for transplantation (*1*). The presence of this type of antibodies is known to be associated with a high incidence of hyper acute or acute-accelerated rejection (*2*). Highly sensitized patients have only a small chance to receive a crossmatch negative donor kidney, because they have developed antibodies against many different HLA antigens resulting from previous contacts with allogeneic cells by pregnancy, blood transfusions, or previous transplants. If no special measurements are taken, these patients will accumulate on the transplant waiting lists. More and more transplant centers are developing strategies to remove circulating HLA alloantibodies in these patients to be able to transplant the patient with a donor kidney despite a positive crossmatch (*3–5*). These desensitization approaches include the use of plasmapheresis, immunoabsorption, intravenous immunoglobulins, and Rituximab. Although excellent short-term results have been described with these approaches, one has to realize that desensitization includes more intensive immunosuppressive treatment associated with side effects related to the nonspecific nature of these immunosuppressive drugs that is a higher incidence of opportunistic infections and cancer. In the international organ allocation program of Eurotransplant (*6*), a different approach is used. The presence of donor-specific antibodies is considered a contraindication for transplantation and special efforts are given to transplant these patients with a crossmatch negative donor. First of all, these patients get priority in the standard Eurotransplant kidney allocation system (*7*). Allocation of kidneys within Eurotransplant is based on a transparent point system and, for every donor, which becomes available in one of the participating countries, patients receive points for among others the degree of HLA match, waiting time, the expected cold ischemia time (distance), and a match prognostic index including the degree of sensitization. Next to this standard allocation program, Eurotransplant has introduced a special program that give the highest priority to highly sensitized patients as soon as a donor becomes available, which is compatible with the patient's antibody profile: the acceptable mismatch (AM) program (*8*). In this review, we describe the past, current, and future situation with respect to the application of an AM program.

DEFINITION OF A HIGHLY SENSITIZED PATIENT

In the past, the degree of sensitization of a patient was based on the percentage panel reactive HLA antibodies (% PRA) in the standard antibody screening. The serum of the patient was screened in complement-dependent cytotoxicity (CDC) against a panel of approximately 50 donors, and depending on the number of positive donors in the antibody screening a percentage PRA was defined. Highly sensitized patients were defined as patients with more than 85% PRA excluding the reactivity of irrelevant autoantibodies. However, this definition is not solid as quality controls have indicated that the percentage PRA is one of the most unreliable markers in histocompatibility testing. External proficiency testing programs within Eurotransplant revealed that the percentage PRA of a particular serum may vary between 10% and 90% (*9*). For this reason, a redefinition of a highly sensitized patient is necessary. More and more centers are introducing a virtual crossmatch, which indicates the degree of sensitization on the basis of the specificities of the HLA antibodies of a patient in relation to the frequencies of the target antigens in the donor population (*10*, *11*). A special tool has been developed on the website of Eurotransplant to define the virtual PRA on the basis of which one can check whether a patient fulfils the criteria of a highly sensitized patient (Fig. 1).

By introducing the antibody specificities detected in the serum of the patient in the computer program, a virtual PRA will be calculated on the basis of the HLA phenotypes of more than 30,000 actual organ donors, which became available during the past 10 years within the Eurotransplant area. In this way, a uniform and more reliable definition of highly sensitized patients is established. Only patients with a virtual PRA more than 85% will be included in the AM program. So far, the virtual PRA is mainly based on HLA-A, -B, and -DR antibody specificities as all donors within Eurotransplant

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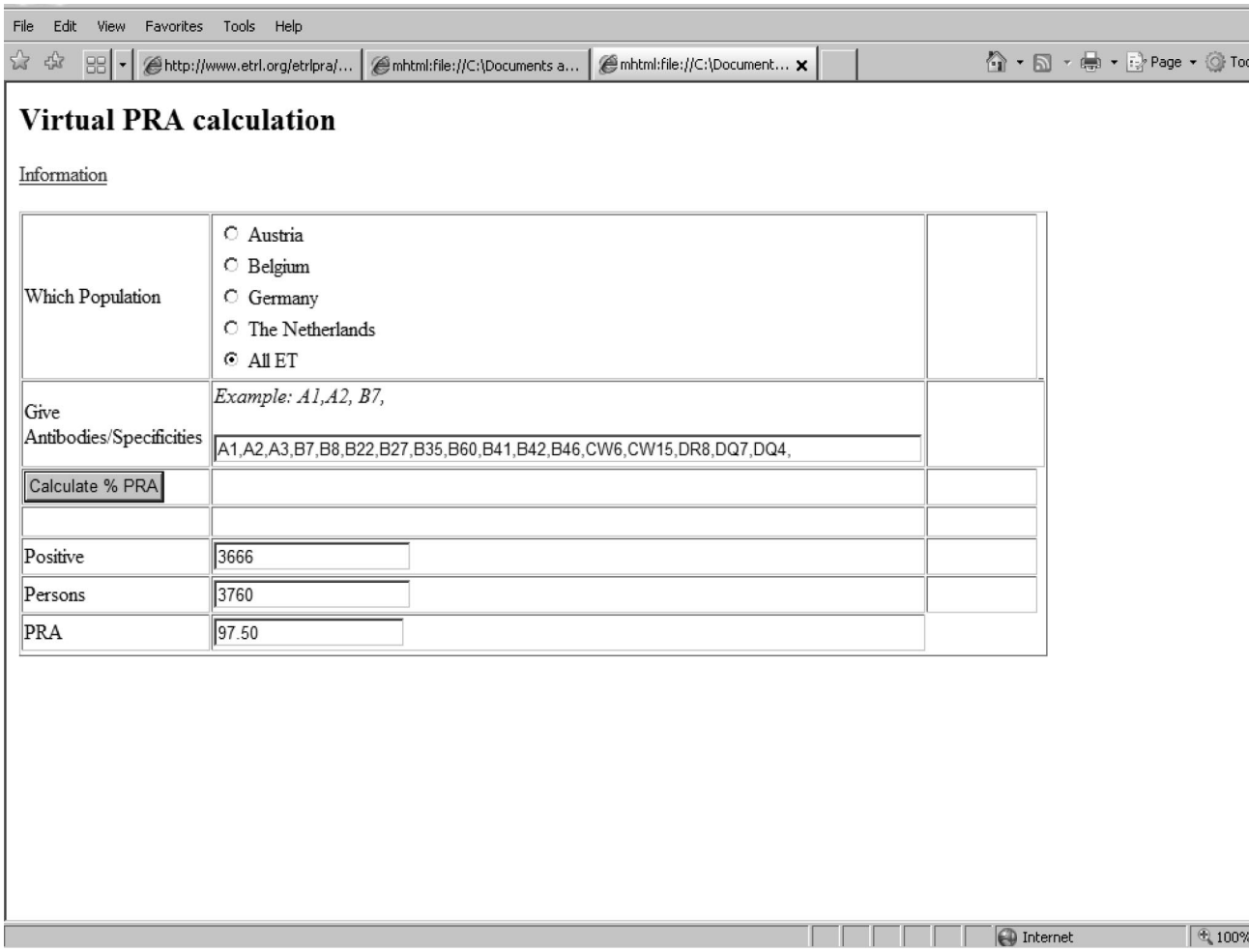

FIGURE 1. Tool to calculate the virtual PRA on basis of the HLA phenotypes of the Eurotransplant donor population.

must be typed for these antigen specificities, whereas HLA-C and -DQ typing is only performed on voluntary basis by some centers.

NEED FOR AN AM PROGRAM

The standard policy in Eurotransplant is registration of the nonacceptable HLA mismatches to prevent selection of donors with HLA mismatches toward which a patient has preformed antibodies. The aim of this policy was prediction and prevention of positive crossmatches in the recipient centers. Kidneys will not be shipped to patients with specific antibodies to the donor. However, the positive identification of all antibody specificities in highly sensitized patients is impossible.

That is the reason why an additional policy has been introduced for highly sensitized patients: the definition of those HLA antigens toward which the patient never formed antibodies. The aim of this approach was prediction of a negative CDC crossmatch.

DEFINITION OF AM

The first indication of AMs comes from the analysis of the HLA types of the panel donors used in the antibody screening. Comparison of the HLA type of those panel donors, which give negative reactions with the serum of the patient, with the patient's own HLA type leads to the identification of acceptable HLA alloantigens. However, this strategy is only useful in patients who have PRA less than 100%. Other approaches include the use of patient specific consisting of blood donors with only one HLA mismatch with the patients who facilitates the definition of AMs. However, for patients with rare HLA types, this approach is not effective as hardly any of such blood donors are available. The main problem with the use of cell-based assays for antibody screening is the fact that cells express different HLA antigens that make it difficult to define AMs. For this reason, the Eurotransplant Reference Laboratory has developed a large panel of single HLA antigen expressing cell lines for antibody screening in highly sensitized patients. By transfecting the cell line K562 with genes coding for the individual HLA class I molecules, cells are available, which express only one single HLA antigen, the so-called single antigen-expressing cell lines (*12*). Screening of a serum against a panel of single antigen-expressing lines will immediately reveal the AMs, which are the HLA antigens expressed on the cell lines that give negative reactions with the serum of the patient. Of course, solid-phase assays, that is ELISA or Luminex, can be used for this purpose as well, but our experience and also the experience of

others is that the conformation of the HLA molecules on a cell is different from that of the isolated HLA molecules present in solid-phase assays.

Especially, the recent observation that natural HLA antibodies can be detected with single-antigen beads in nonsensitized individuals supports this reasoning (*13*). Targets for these natural HLA antibodies are most likely epitopes present on denatured HLA molecules present on the luminex beads. For this reason, we prefer the use of cell-based assays over solid-phase assays. Other approaches that are helpful to define AMs include the analysis of the HLA types of the mothers of the highly sensitized patients because a proportion of highly sensitized patients tend not to make antibodies against the noninherited maternal HLA antigens (*14*).

During pregnancy, the immune system of these patients is probably programmed by HLA antigens of the mothers in such a way that later in life the immune response to the maternal HLA antigens is less aggressive than the immune response to other HLA mismatches including those of the noninherited paternal HLA antigens.

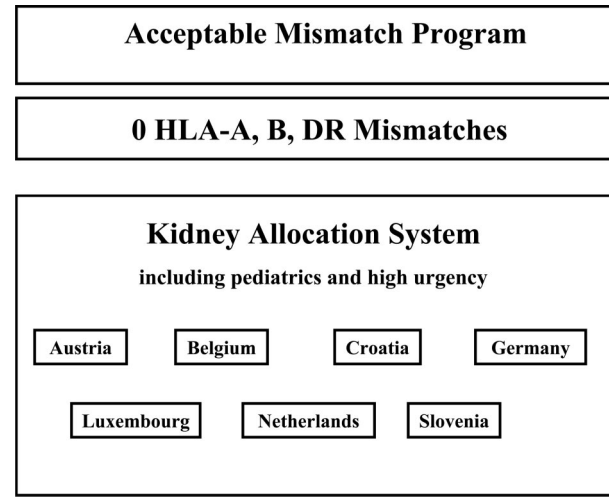

FIGURE 2. Priorities of kidney allocation with Eurotransplant: the acceptable mismatch program has the highest priority.

APPLICATION OF HLAMATCHMAKER FOR THE IDENTIFICATION OF AM

In the past, the identification of AMs was based on the identification of HLA antigens toward which the patient did not make antibodies. However, in the mean time, the amino acid sequences of all HLA alleles are known and specific antibody epitopes have been defined on the different HLA antigens. Some of these epitopes are specific for a particular HLA allele whereas others are shared between different HLA alleles (*15*). Based on this knowledge, it is possible to identify the specific antibody epitopes on an allogeneic HLA molecule and, even more, to predict whether a certain HLA mismatch will be able to induce HLA antibodies in a specific patient. In this respect, a computer algorithm, HLAMatchmaker, has shown to be helpful (*16*, *17*). In this program, HLA antigens are defined as a string of potential antibody epitopes (in the original version consisting of three amino acids, triplets). Some of these potential epitopes are shared between different HLA antigens and may also be present on the HLA molecules of the potential antibody producer. Therefore, an HLA mismatch should be considered in the context of the HLA phenotype of the potential antibody producer. Only those polymorphisms that are not present on the patient's own HLA antigens can lead to a humoral immune response. That is, indeed the concept of the HLAMatchmaker algorithm (*18*). HLA mismatches, which only have triplets that are shared by the different HLA antigens of the antibody producer, will not lead to the induction of HLA antibodies. This theoretical concept was validated by in vitro serological crossmatches in highly sensitized patients. Both in the context of kidney graft rejection and pregnancy, a strong correlation between antibody production and the number of mismatched triplets was found. In the case of a zero mismatch or only a few triplet mismatches, the chance for the patient to be immunized is low (*19*, *20*). For this reason, HLAMatchmaker is routinely used for the identification of potential acceptable HLA mismatches in highly sensitized patients and so far the concept has proven to be effective.

THE USE FOR AM FOR THE ALLOCATION OF KIDNEYS

All acceptable HLA mismatches, which have been identified, will be added to the HLA phenotype of the recipient. It is assumed that a potential kidney donor with an HLA phenotype, which is a combination of the patient's own HLA and one or more AMs, will have a negative CDC crossmatch with the sera of this patient. If such a donor becomes available somewhere in the Eurotransplant area, mandatory shipment of the donor kidney to the recipient center will take place. A final crossmatch will only be performed in the recipient center. Within the Eurotransplant kidney allocation system, the AM program has the highest priority followed by the allocation of kidneys to fully HLA-matched recipients, before the early described point system is applied (Fig. 2).

INCREASED TRANSPLANTATION RATE OF HIGHLY SENSITIZED PATIENTS INCLUDED IN THE AM PROGRAM

As patients within the AM program have the highest priority in the Eurotransplant kidney allocation, it is not surprising that the chance that highly sensitized patients receive a suitable crossmatch negative organ is significantly increased by their inclusion in the AM program. Approximately 60% of the highly sensitized patients will be transplanted within 2 years after inclusion in the AM program. In contrast, patients who benefit only of the extra points in the standard Eurotransplant allocation program will have approximately 20% chance to be transplanted within the same time period. Despite its success, the introduction of the AM program has also led to some ethical discussions within Eurotransplant. The consequence of giving the highest priority to highly sensitized patients is that other patients have to wait longer. Because only 1% of the patient population is highly sensitized, the impact on the waiting time of the other patients is only minor (on average their waiting time will increase with a few weeks). Taking into consideration that before introduction of the AM program many highly sensitized patients were waiting for 10

to 15 years without receiving any proper donor offer, the current policy is accepted by the Eurotransplant community. However, one can imagine that in populations with a much higher incidence of highly sensitized patients or if one would like to include other less-sensitized patients in a similar program, a different conclusion will be made.

GRAFT SURVIVAL OF AM PATIENTS

Inclusion in an AM program clearly facilitates transplantation of highly sensitized patients as shown by the fact that a significant proportion of the AM patients receive a graft in a short time period but according to the literature graft survival in sensitized patients is much worse than in nonsensitized patients (*21*). However, this is certainly not the case in patients transplanted via the AM program.

Previously, we published that highly sensitized patients transplanted via the AM program have the same short-term graft survival as nonsensitized patients within Eurotransplant (*22*). In contrast, other sensitized patients have indeed a significantly poorer graft survival.

The reason for that is probably that in the AM program the exact antibody profile of the patient is known and especially the identification of those HLA mismatches toward which the patient never formed antibodies has been proven over and over again in the laboratory. This is not the case in other sensitized patients for which these extensive laboratory studies have not been performed. These patients are transplanted on the basis of a negative crossmatch, not supported by hard laboratory data showing that the donor HLA mismatches are indeed acceptable. If such a policy will be introduced for all sensitized patients, one can assume that these graft survival will improve as well. Not only the short-term graft survival is excellent but also the long-term graft survival in these AM patients is excellent and similar to that of nonsensitized patients (Fig. 3). The degree of HLA matching in the AM cohort is comparable with that of the total Eurotransplant patient population (Table 1). No significant effect of HLA-A, -B, and -DR matching was observed in patients transplanted via the AM program.

WHICH ANTIBODIES ARE RELEVANT?

So far, the Eurotransplant AM program has only been used to predict a negative CDC crossmatch with all relevant sera of a patient. This means that antibody reactivity is mainly based on the standard CDC assay. The reason for this policy is that it is generally accepted that a positive CDC crossmatch is

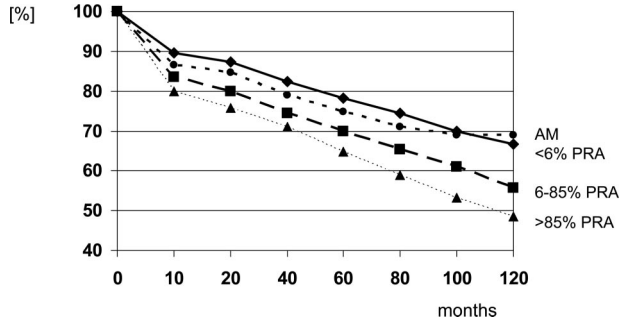

FIGURE 3. Long-term graft survival of patients transplanted via the AM program.

TABLE 1. Comparison of the degree of HLA matching between the AM and the ET cohort

	No. HLA Mismatches					
	AM (%)			Total ET (%)		
	0	1	2	0	1	2
HLA-A	50	38.7	11.3	38.1	49.5	12.4
HLA-B	34.7	50	15.3	30.9	57.9	11.2
HLA-DR	67.3	32	0.7	56.3	42.4	1.3

HLA, human leukocyte antigen; ET, Eurotransplant; AM, acceptable mismatch.

a contraindication for transplantation. In the meantime, sensitive antibody screening assays have become available, mainly taking advantage of solid-phase assays in which antibodies react with isolated HLA molecules in ELISA or in flow (*23*, *24*). Especially, Luminex-based assays have gained a lot of popularity among the HLA laboratories (*25*, *26*). Although these techniques are certainly more sensitive than CDC, the clinical relevance of the antibodies detected is not clear. Nevertheless, many centers are applying these assays already routinely for their patient care and patients are not transplanted, or desensitized, when noncomplement fixing donor-specific antibodies only detectable in Luminex are present. To check the possible clinical relevance of donor-specific antibodies, only detectable in Luminex, a retrospective analysis was performed by the group of Van den Berg-Loonen et al. (*27*). They reanalyzed the original peak sera of patients transplanted in the AM program only based on a negative CDC crossmatch by single-antigen Luminex beads. Patients who had donor-specific antibodies in Luminex had a higher incidence of acute graft rejection in the first 6 months after transplantation (biopsy proven) but graft survival was similar in patients with and without donor-specific antibodies in Luminex.

This led to the preliminary conclusion that patients with donor-specific HLA antibodies detected by Luminex only tend to undergo more acute rejections but these antibodies do not have a detrimental effect on graft survival. Of course, confirmation in a larger patient group is necessary before final conclusions are drawn, but it is clear from this and many other studies (*28–30*) that the presence of donor-specific antibodies in Luminex does not have the same detrimental effect as donor-specific antibodies detectable by CDC. Donor-specific antibodies in Luminex may be considered a risk factor but are certainly not a contraindication for transplantation (*1*, *31*). That is the reason why so far within Eurotransplant the AM program is only available for patients with CDC antibodies. Future studies are necessary to reveal which subpopulation of antibodies, detectable in Luminex (possibly dependent on antibody titer, immunoglobin class, or kind of sensitization), have a similar detrimental effect as CDC antibodies. Only these relevant antibodies should be taken into consideration in the decision whether a patient should be included in the AM program. Without this restriction, the exclusivity of the program would change enormously as many patients will end up to be highly sensitized if all positive reactions in Luminex are considered relevant. Recent adaptations of the luminex antibody detection such as monitoring of

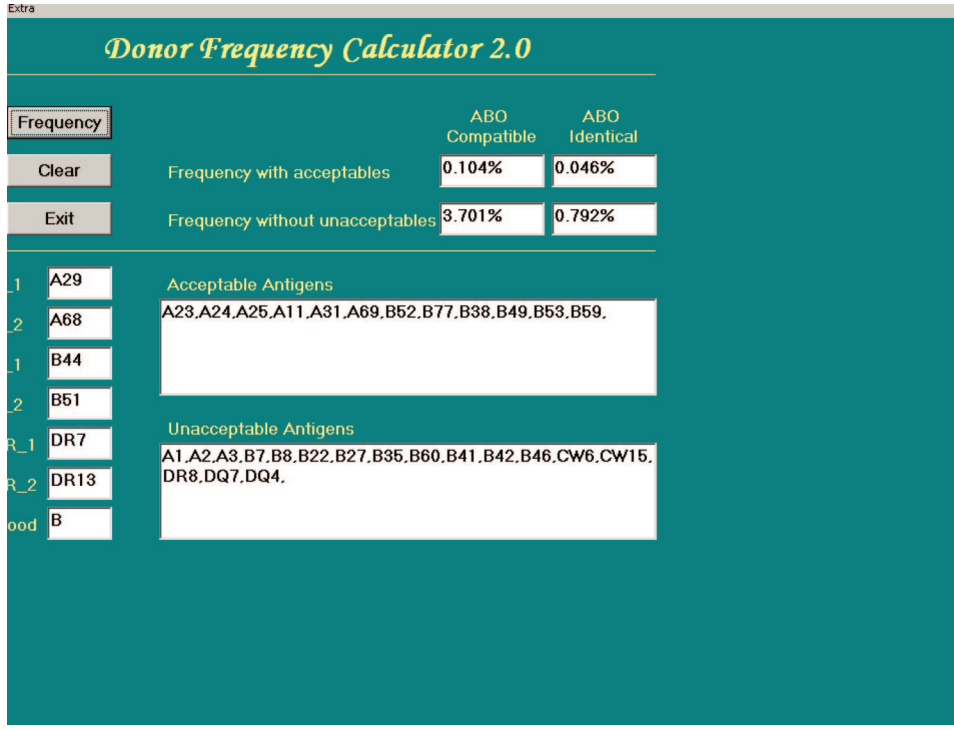

FIGURE 4. Website-based tool to calculate the chance that a compatible donor will become available for a patient included in the AM program.

C4d-fixing antibodies (*32*) or IgG subclasses (*33*) may be useful in this perspective.

OTHER OPTIONS

The AM has proven to be a useful tool to transplant a large proportion of the highly sensitized patients in a short period of time. However, approximately 40% of the highly sensitized patients cannot be helped by the current procedure because no compatible donor will become available within the Eurotransplant population. A special tool has been developed on the Eurotransplant website to estimate the chance that a patient will be transplanted within the AM program (Fig. 4).

After introducing the patient's own HLA antigens and the AMs, the program will calculate the chance that a compatible donor will be available within the Eurotransplant donor population. On the basis of this information, the clinician can decide whether it is worthwhile to wait until the patient will get a transplant via the AM program or whether alternative approaches should be used. At the moment, the only available alternatives are desensitization or for a limited number of patients in the Netherlands the National Paired Donor exchange program (*34*).

However, to our opinion, there is a good opportunity to create an additional option for these patients. Based on the success of the Eurotransplant AM program, similar programs have recently been implemented in France and Greece, whereas implementation is in progress in Scandia transplant, Switzerland, and even Canada. It is to be expected that in the near future many more countries within Europe will implement a similar approach. This would open the possibility to introduce a common solution for patients who cannot be transplanted within the own allocation program. The alternative that we have in mind and that is currently investigated is the setup of an AM program Europe wide. Patients for whom the AMs have been determined but who cannot be transplanted in a reasonable time period within the local allocation program will be registered on one common European waiting list. When somewhere within Europe a donor becomes available, which is compatible with the HLA profile of an AM patient, the kidney should be mandatorily shipped to the recipient center, similar to the current protocol within Eurotransplant. This would mean an enormous extension of the donor population, and especially of the number of HLA phenotypes within the donor population, and related to that a significantly increased chance that one of these donors is compatible with a highly sensitized patient. The logistics of such an effort is a challenge but will be investigated in the near future. The long-lasting experience of Eurotransplant with international organ allocation will be certainly of benefit for the set up of such an exercise.

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