Original Article

Rejection Prophylaxis in Corneal Transplant

A Randomized Study of HLA Matching (The FANCY Study)

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Summary

Background: Graft survival after corneal transplant is threatened by immunological graft rejection. Twenty to thirty percent of patients with corneal transplants experience at least one rejection episode in the first 5 years after transplantation. Prophylaxis through matching for human leukocyte antigens (HLA) is controversial. We herein report the results of the Functional ANTigen matChing in keratoplastY (FANCY) trial.

Methods: FANCY was a randomized, double-blind, multicenter clinical trial. The primary objective was to evaluate superiority of HLA matching versus random graft assignment. The primary endpoint was rejection-free graft survival. We included both normal-risk and high-risk indications. The study is registered with ClinicalTrials.gov (NCT00810472).

Results: 721 patients were included, 639 patients were randomized. 474 patients underwent keratoplasty within the study; 165 patients received grafts outside the trial. One patient died and one patient was lost to follow up. We observed 33 graft rejections in the HLA matching arm ($n = 224$). The corresponding estimated cumulative incidence rate of immune reactions after two years was 15.7%. We observed 40 rejections in the control arm ($n = 249$). After two years this yields an estimated cumulative incidence rate of 17%.

Conclusion: In our heterogenous study group, HLA matching did not show a significant advantage compared to random graft assignment. The rejection rate in our sample was lower than expected. Therefore no definite conclusions can be drawn as to whether HLA matching is beneficial in corneal transplantation.

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All diseases affecting the shape or transparency of the cornea are potentially vision threatening. Corneal disease is among the most common causes of blindness (1). In Germany, Fuchs endothelial dystrophy, bullous keratopathy, keratoconus, and corneal scars are the most prominent indications for corneal transplants. For more than a hundred years now, keratoplasty has been the most commonly performed transplantation procedure worldwide (2).

Background

The ocular immune privilege

The success of keratoplasty is owed to a phenomenon known as the ocular immune privilege: Allogeneic material is tolerated in the anterior eye chamber whereas it is vigorously rejected in almost all other body locations (3). The immune privilege of the eye is attributed to sequestration behind an efficient blood–retina barrier, local humoral immunosuppression, and the anterior chamber-associated immune deviation (ACAID). ACAID is maintained by antigen-specific antigen-presenting cells that migrate from the eye and induce specific regulatory T cells that systemically suppress graft rejection (4).

Graft rejection

However, despite regular prophylaxis with topical steroids, 20 to 30% of patients with corneal transplants experience at least one rejection episode in the first 5 years after transplantation (2, 5, 6). Whenever the non-regenerative graft endothelium is immunologically destroyed, the graft fails, either directly or later on. Graft rejection thus threatens the long-term success of the keratoplasty procedure (7). Repeat keratoplasty carries an elevated risk of graft rejection, irrespective of the primary indication (8). This is relevant because every single keratoplasty comes with a certain risk of blindness and the number of keratoplasty procedures that can be performed per eye is limited.

The situation is worse in eyes with compromised immune privilege. This occurs as a result of trauma, chronic surface inflammation, or due to the patient requiring an oversized graft. In these “high-risk” situations, almost all grafts are rejected in the first 3 years after transplantation, despite topical and systemic immunosuppression (9). One reason may be that long-term adherence to systemic immunosuppressive

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TABLE 1

Overview of the evidence on HLA matching in human keratoplasty

First author/study acronym (reference)	Year of publication	Number of patients	HLA loci under investigation	Evidence level	Remarks
Boisjoly (18)	1990	438	A, B	II	Beneficial effect predominantly in low-risk constellations
CCTS (13, 14)	1992	419	A, B/DR/AB0	I	No beneficial effect, high-risk constellations
Hoffmann (19)	1994	248	A, B/DR	II	Beneficial effect both in low- and high-risk constellations
CTFS (20)	1997	602	A, B/DR	II	HLA class I (A/B) matching beneficial, HLA class II (DR) matching detrimental
Munkhbat (21)	1997	81	DR, DQ, DP	II	HLA-DP matching beneficial, no effect for HLA-DR and HLA-DQ, low- and high-risk constellations
Baggesen (22)	1996	74	DR/DQ	II	Beneficial effect for HLA class II (DR), high-risk constellations
Völker-Dieben (23)	2000	1 681	A, B/DR	II	Beneficial effect of HLA class II (DR) matching against a background of moderately or well matched HLA class I, low- and high-risk constellations
Khairuddin (24)	2003	459	A, B/DR	II	Beneficial effect in both low- and high-risk constellations
Bartels (25)	2003	303	A, B	II	Beneficial effect in high-risk constellations
Reinhard (26)	2004	48	A, B/DR	II	Beneficial effect in high-risk constellations
Reinhard (27)	2004	418	A, B/DR	II	Beneficial effect in low-risk constellations
Böhringer (28)	2010	1 561	A, B/DR	II	HLAMatchmaker, beneficial effect in well-matched HLA class I (A/B), additional interaction between HLA class II (DR) and HLA class I in low- and high-risk constellations

HLA, human leukocyte antigen

medications such as cyclosporine A or mycophenolate mofetil is substantially limited (10).

HLA histocompatibility

Matching for human leukocyte transplantation antigens (HLA) has a key role in bone marrow and kidney transplantation (11). In keratoplasty, however, HLA matching is not performed widely (12).

This is, at least partially, due to controversial evidence. *Table 1* summarizes our search of Pubmed using the keywords “keratoplasty AND HLA AND matching”. Only two of the retrieved publications reported on prospective trials, with the collaborative corneal transplant study (CCTS) being the only randomized controlled trial (13). The CCTS did not detect a beneficial effect of HLA matching in keratoplasty. This result was later questioned, however, after errors in HLA typing were discovered (14). The other prospective trial, the corneal transplant study II (CTFS II), did not assess the efficacy of HLA matching per se, but the influence of HLA class II matching on allograft rejection in patients well matched at HLA class I (15).

A search of two clinical trials registries (<https://clinicaltrials.gov/> and <http://apps.who.int/trialsearch/>) using the same above-mentioned keywords yielded no further trials.

HLA matching has been questioned after animal experiments using mouse models have shown that the

“minor” (H) transplantation antigens may be more important in graft rejection than the major histocompatibility complex (16, 17, *Table 1*).

Open questions

It is currently unclear whether HLA matching is effective in rejection prophylaxis in human keratoplasty. It is also unexplained whether H transplantation antigens play a comparably significant role in human corneal transplantation as they do in animal models. We designed the Functional ANtigen matChing in keratoplastY trial (FANCY) to answer both questions.

Methods

Study design

The study protocol of the FANCY trial has been published elsewhere (29). FANCY was a randomized, controlled, double-blind, multicenter clinical trial with two parallel arms. We included all patients scheduled for penetrating or lamellar endothelial keratoplasty. Patients with deep anterior lamellar keratoplasty were excluded. Another major exclusion criterion was an anticipated waiting time for a matching donor of more than 6 months. This was predicted based on the rareness of patients' HLA phenotypes (30).

More methodological data including statistical analyses and sample size calculation can be found in the *eMethods* section.

FIGURE 1

CONSORT
flow chart.
CONSORT,
Consolidated
Standards of
Reporting Trials;
HLA, human
leukocyte antigen

Results

We included a total of 721 patients. 68 patients were excluded due to rare HLA phenotypes and 14 for other reasons. 639 patients were randomized. 165 patients withdrew early from the trial and were not followed (5 were found in retrospect not to have met the inclusion criteria, one patient died, 97 withdrew their informed consent while on the waiting list, and 62 patients dropped out for other reasons). 474 patients underwent keratoplasty (337 received penetrating keratoplasty, 13 limbo-keratoplasty, and 123 lamellar endothelial keratoplasty). Only one patient was lost to follow-up. A CONSORT flow diagram showing the flow of participants through the trial is provided in *Figure 1* (CONSORT, Consolidated Standards of Reporting Trials).

Primary endpoint

473 patients received keratoplasty as part of the FANCY study and were followed for at least one day. The baseline characteristics of the intention-to-treat (ITT) population (study arms as randomized, irrespective of actual treatment) are summarized in *Table 2*. For 39 patients in the HLA matching arm no suitable donor was found within the study period. These patients received transplants from the next suitable donors. The actual degrees of HLA matching are given in *eTable 1*.

We observed 33 graft rejection episodes and 19 graft failures in the matching arm ($n = 224$). The corresponding cumulative incidence rate of immune reactions after two years is 15.7% (95% confidence interval: [11.4; 21.5]). In the control arm, we observed 40 rejection episodes and 14 graft failures ($n = 249$). This yields a cumulative incidence rate of 17% [12.7; 22.8] after two years. The Fine and Gray model that compares the cumulative incidence rates of graft rejection in the ITT population shows an estimated subdistribution hazard ratio of 0.97 [0.61; 1.54], $p = 0.90$. The incidence rates of immune reactions in the ITT population are shown in *Figure 2*.

Sensitivity analyses

The as-treated (AT) population comprises 386 patients with complete HLA-typing from the reference laboratory. We subdivided the AT population on the basis of actual HLA mismatches into a well-matched group (up to 2 out of 6 possible HLA mismatches at the HLA loci A, B and DR) and an unmatched control group (between 3 and the maximum of 6 HLA mismatches at the loci A, B and DR). To foster comparability with the earlier studies, HLA typing was performed at low resolution ("broad alleles").

As a third group, we also included in the AT population patients without complete HLA-typing. No

TABLE 2

Baseline characteristics of the intention-to-treat population

Study arm	Random (n = 249) n (%)	Match (n = 224) n (%)	Total (n = 473) n (%)
Male sex	131 (52.6)	122 (54.5)	253 (53.5)
Caucasian ethnicity	244 (98.0)	221 (98.7)	465 (98.3)
Stromal neovascularization	27 (10.8)	21 (9.4)	48 (10.1)
Previous graft rejection in study eye	15 (6.0)	14 (6.3)	29 (6.1)
Lamellar endothelial keratoplasty	59 (23.7)	64 (28.6)	123 (26.0)
	Median (range)	Median (range)	Median (range)
Age [years]	66 (18–90)	65 (19–88)	66 (18–90)
Follow-up [days]	721 (4–985)	721 (4–1028)	721 (4–1028)

FIGURE 2

Cumulative incidence of graft rejection

Cumulative incidence of immune reactions in the "intention-to-treat" population; graft rejection rates were comparable in both study arms

clinically relevant differences were observed in the AT population (eTable 2, eTable 3).

For high-resolution matching we also conducted an HLA-Matchmaker analysis. We dichotomized the patients on the basis of 10 mismatched HLA-Matchmaker eplets both for HLA class I (A and B) and class II (DR) separately. This threshold was chosen to achieve group sizes comparable to the conventional HLA matching. We assessed the degree of HLA class

I matching against a background of HLA class II matching as this had been specifically targeted in the CTFS II (15). To this end, we formed 4 subgroups:

- Subjects well-matched both at HLA-class I and HLA-class II,
- Subjects well-matched only at HLA-class I,
- Subjects well-matched only at HLA-class II
- Subjects neither matched at HLA-class I nor at HLA-class II.

On this basis we observed a weak beneficial tendency for matching at HLA-DR in HLA-class I matched grafts. However, this tendency was not statistically significant: testing with a Fine and Gray regression model yielded a p-value of 0.58. A comparison of the subgroups AB <10, DR <10 versus AB ≥ 10, DR ≥ 10 yielded a p-value of 0.40.

Minor histocompatibility antigens

We typed donors and recipients for the minor H antigens HA-3, HA-8, ACC-1, and ACC-2 as these have been identified to be of importance in bone marrow transplantation and graft-versus-host disease (GvHD) (31). Immunological constellations were rare. Therefore, these antigens are of limited clinical use in keratoplasty patients (Table 3). In the FANCY trial, no gender difference in graft rejection rates was observed for women receiving a man's cornea. The H-Y effect was not stronger when the HLA-A1 and A1 restrictions were taken into account (data not shown).

Discussion

HLA matching as standard treatment

FANCY was designed in 2007. At that time, penetrating keratoplasty was the undisputed gold standard. Graft rejection was the major barrier towards graft survival. FANCY was designed to address the question whether HLA matching should be introduced as a standard treatment. Therefore, we deliberately included the low-risk situations (e.g. keratoconus and Fuchs endothelial dystrophy). It is important in patients with low-risk indications to limit their waiting time for a matching donor, because the risk for and the impact of graft rejection is low compared to high-risk indications. For ethical reasons, we limited the waiting time to 6 months because the delay of surgery had to be balanced against the putative benefit from HLA matching. To achieve this, we assigned the next available graft whenever a patient waited longer than 6 months. However, we maintained the randomized group assignment irrespective of the actual graft assignment. This intention-to-treat approach therefore enabled assessment of the usefulness of HLA matching within a maximum waiting time of 6 months. We found that HLA matching was not clearly beneficial under these circumstances. Interestingly, a total of 97 patients withdrew informed consent while on the waiting list. Unfortunately, we did not collect data on the individual motivations for these decisions. We hypothesize, however, that a prominent reason may have been the desire to schedule the procedure instead of having to wait for an indefinite time in the trial.

TABLE 3

Distribution of the immunologically relevant minor (H) antigen mismatches *

H Locus	Restricting HLA allele(s)	% Donors positive for restricting HLA allele(s)	% Mismatched (non-shared hypothesis), absolute numbers in parentheses	% Difference in rejection frequency after 2 years (matched versus mismatched)
HA3	HLA-A1	20%	14% (55)	-3%
HA8	HLA-A2	39%	14% (55)	-2%
ACC2	HLA-B44	22%	3% (11)	Group sizes too small
ACC1C	HLA-A24	19%	12% (45)	Group sizes too small
ACC1Y	HLA-A24	19%	6% (21)	+1%
H-Y	HLA-A1/HLA-A2	66%	24% (89)	-2%

* With the exception of the H-Y antigens, the sparsity of immunological constellations limits clinical relevance
HLA, human leukocyte antigen

The efficacy of HLA matching

The negative result with respect to the primary endpoint is unexpected because several retrospective publications report a benefit from HLA matching (*Table 1*). This may be attributable to our intention-to-treat approach by which patients randomized into the HLA matching arm who had been waiting in vain for a matching donor for 6 months were allocated the next available random graft. We therefore conducted an “as-treated” analysis. This analysis too showed no advantage of HLA matching in preventing immune reactions (see *eMethods*). However, analysis using HLAMatchmaker revealed that transplant recipients’ immunological risk is increased in situations where donor materials are well-matched at HLA class I but mismatched at HLA class II. This is in accordance with a large retrospective study on the influence of class II matching against a background of histocompatibility at HLA class I in penetrating keratoplasty (28).

One reason for the negative outcomes may be that FANCY is underpowered, considering that substantially fewer cases of graft rejection were observed than had to be expected. No evidence of underreporting was documented in the on-site monitoring reports. The degree of immunosuppression was not higher than expected (data not shown). The most likely explanation for this is the remarkable success of the posterior lamellar techniques, particularly of Descemet membrane endothelial keratoplasty (DMEK). This method was introduced during the recruitment phase of FANCY. However, the reduced risk of graft rejection in DMEK grafts was not recognized until mid-2012 (32), when patient enrollment in FANCY was completed and 33 patients with DMEK had been included. However, we did not see a stronger matching effect in a subgroup analysis of only penetrating keratoplasties, with the caveat of a lowered statistical power (data not shown).

While most retrospective studies with positive results were larger than FANCY, some smaller studies

also reported a beneficial effect of HLA matching (*Table 1*). These smaller studies, however, might be confounded by factors such as the surgeon’s learning curve or by bias from HLA matching being offered to a selected group of patients only.

Taking into consideration the above-mentioned statistical power considerations, it is still possible that HLAMatchmaker-based HLA matching might be beneficial in keratoplasty. An HLAMatchmaker-based approach in particular might help avoid unfavorable constellations with respect to graft tolerance induction.

Minor (H) antigens in human keratoplasty

Alternatively, the lack of effect from HLA matching could be due to a lack of biological relevance of HLA antigens in keratoplasty. Accordingly, minor (H) transplantation antigens have been associated with graft rejection in mouse models of keratoplasty, whereas this is not the case for the major histocompatibility complex (33, 34). A recent genome-wide analysis has linked corneal graft rejection to several minor loci in a swine keratoplasty model (16). Minor antigens have also been studied in human keratoplasty (35). In particular, H-Y antigens have been assessed in more detail. A recent meta-analysis has shown that grafts from male donors represent an immunological risk factor in female patients (36).

In the FANCY trial, we opted to analyze ACC-1, ACC-2, HA-3, and HA-8 in addition to the H-Y antigens. This choice was based on their importance in bone marrow transplantation. Our data suggest that the immunological role of the minor antigens ACC-1, ACC-2, HA-3, and HA-8 in keratoplasty is subordinate. Their apparently small effect on graft rejection and the sparsity of the mismatched constellations limit their clinical relevance (*Table 3*). Interestingly, FANCY did not confirm the positive findings of the H-Y meta-analysis (36). One explanation might again be that the statistical power is lower than anticipated.

Key messages

- Keratoplasty can often cure vision loss due to corneal disease.
- The success of corneal transplantation is threatened by immune reactions.
- The evidence for HLA matching in rejection prophylaxis is controversial.
- The FANCY (Functional Antigen Matching in Keratoplasty) study is the largest randomized trial to date to investigate HLA matching in keratoplasty.
- The FANCY study found no clinically significant advantage of HLA (human leukocyte antigen) matching. However, the rejection rate was lower than expected.

By comparison, the incidence rate of graft failure due to rejection in the CCTS, for example, was as high as 26% after 3 years (13).

Conclusion

FANCY is the largest clinical trial on HLA matching in keratoplasty with high resolution HLA and minor-antigen typings. Our major finding is that HLA matching is not beneficial as a standard treatment in a mixed group of patients undergoing normal-risk or high-risk keratoplasty.

However, the study is underpowered due to an event rate that was lower than anticipated. This renders definite conclusions impossible. It is still possible that HLA matching is of benefit in selected patients, provided they are willing to wait for a matching donor.

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Conflict of interest statement

The authors declare that no conflict of interest exists.

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► **Supplementary material**
eReferences:
www.aerzteblatt-international.de/ref1518
eSupplement, eTables:
www.aerzteblatt-international.de/18m0259

CLINICAL SNAPSHOT

Non-Uremic Calciphylaxis

A 76-year-old man suddenly developed painful necroses and net-like areas of erythema on the left lower leg (*Figure a*). The initial differential diagnosis included peripheral artery disease (PAD), coumarin necrosis, and vasculitis. Seven weeks later, after compression therapy had been tried unsuccessfully and PAD had been ruled out, he was referred to our dermatological clinic. Histological examination revealed calcification in arterioles at the dermal/subcutaneous interface (*Figure b*), as well as in adipose tissue, consistent with the diagnosis of non-uremic calciphylaxis.

The serum calcium and phosphate levels and the creatinine clearance were unremarkable, and the parathyroid hormone level was only minimally elevated. We initiated both intravenous and topical

sodium thiosulfate treatment, and the lesions healed completely in seven months. Calciphylaxis is a rare condition with high mortality. Most of the affected patients have advanced chronic renal failure and need dialysis. It is less well-known that calciphylaxis can also arise in patients with normal renal function. Coumarin use, obesity, diabetes mellitus, and mildly impaired renal function are the main risk factors for non-uremic calciphylaxis. Its pathogenesis is not yet well understood.

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Translated from the original German by Ethan Taub, M.D.

a) Necrosis and net-like areas of erythema on the left lower leg
 b) Calcification in arterioles at the dermal/subcutaneous interface

Supplementary material to:

Rejection Prophylaxis in Corneal Transplant

A Randomized Study of HLA Matching (The FANCY Study)

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eMETHODS

Graft allocation

Blood samples from all graft recipients were continuously sent by the study centers to the reference laboratory. HLA data (HLA, human leukocyte antigen) were entered into the trial web portal in a timely manner. In the HLA matching arm during the first three months, the study matching algorithm sought for the first graft with less than 3 (out of 6) mismatched HLA alleles. More mismatches were allowed thereafter (epitope mismatches determined by HLAMatchmaker) (e1). After 6 months of futile waiting, the next available graft was allocated regardless of HLA mismatches. In the control arm, the study matching algorithm always allocated the next available graft for which there was no matching recipient in the HLA matching arm. All HLA data and the degree of matching were concealed from the investigators and patients throughout the trial.

Grafts

All grafts were procured locally at the centers and stored in organ culture as recommended in the EEBA guidelines (EEBA, European Eye Bank Association) (e2). Grafts were not exchanged between centers. The patients from smaller centers without a cornea bank were registered on behalf of the center with Bio Implant Services (NL). The patients were either registered for a matched or unmatched graft depending on the study arm. In order to maintain blinding in investigators and patients to trial arm allocation, this was done centrally by the study coordinator. Grafts from this source were neither H- nor HLA-typed in the reference laboratory. However, HLA typing from Bio Implant Services was compatible with the FANCY trial. This affected 17 patients from the HLA matching arm and 19 from the control group. In cases of technical failure during surgery, the use of replacement grafts was permissible even if these had not been H- and HLA-typed in the reference laboratory. We assume that missing data of minor antigens due to this can be viewed as missing at random.

Group assignment

The randomization list was produced off-site by the clinical trials centre in Freiburg with a computerized random number generator. The random sequence was securely deposited in the trial software package. The blood samples for HLA typing were collected during the enrollment visits and sent to the reference laboratory. From there, all HLA data were entered into the web-based trial portal. All eligible patients were automatically randomized into either the matching arm or the control arm as soon as the HLA-data had been entered. Group assignment, randomization sequence and HLA-data were thus concealed from the investigators and patients.

HLA matching and allocation strategy

We opted for a matching strategy in line with the majority of previous investigations in the field (*Table 1*). Therefore, we considered only the loci HLA-A, HLA-B and HLA-DR. We compared the alleles from donor and recipient at low (“broad”) serological typing resolution. We considered a keratoplasty well matched when the donor featured at maximum of two different HLA-alleles that were not present in the recipient with no prioritization of HLA-loci. After 5 months of futile waiting, donors with a favorable HLAMatchmaker profile (as described in [e1]) were additionally accepted. After 6 months, the next available graft was assigned. In the control arm, the next available donor was assigned. However, the HLA matching arm was always prioritized to make sure that the group assignment was not revealed by short waiting periods.

Follow-up

For regulatory reasons, we did not specify the medical treatment in the trial protocol. We rather advised the investigators to adhere to the recommendations of the “Sektion Kornea” of the German Ophthalmological Society whenever feasible (e3). We scheduled a total of 4 postoperative follow-up visits in two years. The patients were advised to present at the transplantation center as soon as possible in case of signs of an immune reaction. Graft rejection was defined as follows:

- Newly diagnosed keratic precipitates with or without graft edema and/or
- Subepithelial infiltrates not explained by preceding adenoviral conjunctivitis.

This had to be confirmed by the principle investigators at the study centers. We additionally collected a slit lamp photograph of the graft at the time of presentation at the study center.

Statistical analyses

The primary endpoint was time from keratoplasty to first endothelial graft rejection.

The sample size calculation was based on an anticipated rejection rate of 30% after 2 years. We presumed that in the HLA matching group 30% of rejection episodes could be prevented. This corresponds to a hazard ratio of 1.51. On this basis, a total of 184 events are needed to detect this difference with a power of 80% at a two-sided significance level of 5%. With accrual period and reference time both set to 1.5 years, a total of 620 patients are required. We validated by means of statistical simulation that this still holds if 10% of patients in the HLA matching arm received a random graft after 6 months. We set the total number of patients to be screened to 720 in order to compensate for the screen failures due to an expected waiting time of more than 6 months and dropouts for other reasons.

According to the intention-to-treat (ITT) principle, the primary analysis is based on the full analysis set (FAS). This means that the patients were analyzed in the treatment arms to which they were randomized regardless of which treatment they actually received. Patients not receiving keratoplasty were excluded from the FAS, as they cannot contribute any information on the primary endpoint.

For sensitivity analyses, we defined an “as-treated” (AT) population as follows: the AT population is a subgroup of the FAS, consisting of those patients who received a typed graft. Patients in the AT population were classified according to the actual number of mismatches. All untyped “non-trial” grafts were included as a third group. The “matching” group comprised of patients with 0–2 HLA mismatches, the “mismatch” group consisted of patients with 3 or more HLA mismatches. For high-resolution matching, we additionally opted for the HLAMatchmaker method (e4).

As outlined in the study protocol, we had originally planned to use a Cox proportional hazards regression model in the intention-to-treat population for primary analysis. However, in the course of the trial, new surgical techniques (such as descemet membrane transplantation, DMEK) have been introduced. These technically challenging methods induced a higher risk of nonimmunologic graft failure. Therefore, we decided to use a Fine and Gray regression model to incorporate this type of graft failure as a competing event (e5). Results are presented as subdistribution hazard ratios (SHR). We used the Aalen–Johanson estimator for the estimation and graphical presentation of cumulative incidence rates of graft rejection and graft failure (e6).

As preplanned, regression models were adjusted for study center, combining centers with less than 5 patients. Regression models were adjusted for age at surgery in additional sensitivity analyses.

Laboratory analyses

Unambiguous high resolution typing was performed on amplified DNA in both forward and reverse directions,

using the GenDx HLA-A, -B, and -DRB1 kit, following the recommendations of the manufacturer. Purified sequencing products were electrophoresed using a 3730 DNA-Analyzer and sequences were analyzed using the SBTengine software. Minor (H) typings were performed retrospectively in the same laboratory as described in an earlier publication (e7).

Ethical considerations

Patient’s written consent was obtained prior to any study-specific procedures. The study received appropriate ethics committee approvals from the master committee (University of Freiburg, 229/07) and the local committees (University of Erlangen–Nürnberg, 4093-CH; University of Duisburg-Essen, 09–4134; University of Frankfurt, 234/09; Medical Association of Hamburg, MC-239/09; Medical Association of Saarland, 134/09; University of Kiel, B 255/09; Medical Association of Rheinland-Pfalz, 837.343.09 (6849); Ludwig-Maximilian-University of München, 294–09; University of Münster, 2009–347-b-S; University of Würzburg, 31/10). We registered the FANCY trial with clinicaltrials.gov (NCT00810472).

Monitoring

The trial was continuously supervised by an external data monitoring committee. Source data verification was performed by clinical study monitors on a regular basis.

Results of the “as treated” sensitivity analysis

In the HLA matching arm ($n = 187$) we observed 26 graft rejection episodes and 18 graft failures. The corresponding cumulative incidence rate of immune reactions after two years is 14.7% (95% confidence interval: [10.3; 21.0]). In the control arm ($n = 199$) we observed 35 rejection episodes and 13 graft failures. This yields a cumulative incidence rate of 18.3% [13.4; 25.0] after two years. The Fine and Gray model that compares the cumulative incidence rates of graft rejection in the per protocol population shows an estimated subdistribution hazards ratio of 0.88 [0.52; 1.47].

eTABLE 1

Degree of HLA matching achieved in the two study arms

Randomized intervention	Total number	Number with complete HLA data from donor and recipient	Minimum	Median	Mean	Maximum
No intervention (drop out)	82	2	2	3	3.00	4
Random	319	200	2	4	4.53	6
Match	320	190	0	2	2.63	6

HLA, human leukocyte antigen

eTABLE 2

Cumulative rejection rates in the as-treated population*

Group	Cumulative rejection rate (%)	95% confidence intervals
Well matched	14.9	[9.8; 22.6]
Unmatched	17.5	[13.2; 23.3]
Matching unknown	15.3	[9.1; 25.9]

* actual HLA compatibility by treatment group; HLA, human leukocyte antigen

eTABLE 3

Cumulative graft failure rates in the as-treated population*

Group	Cumulative failure rate	95% confidence intervals
Well matched	9.9	[5.9; 16.6]
Unmatched	7.4	[4.7; 11.5]
Matching unknown	3.2	[0.8; 12.4]

* actual HLA compatibility by treatment group; HLA, human leukocyte antigen