Supplementary Material for:
Clinical and molecular profiling to develop a potential prediction model for the response to alemtuzumab therapy for acute kidney transplant rejection

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Table of Contents:

1. Detailed methods of gene expression profiling .................................................. 2
2. TRIPOD checklist ......................................................................................... 3-4
3. Supplementary Tables:
   Table S1 Collected baseline characteristics of the study population ............... 5
   Table S2 Shrinkage of regression coefficients in the lambda.min LASSO model ........ 6
   Table S3 Shrinkage of regression coefficients in the lambda.1SE LASSO model .......... 7
   Table S4 Bootstrap variable selection ............................................................... 8
   Table S5 Logistic regression model: Included variables and statistics .......... 9
   Table S6 Baseline characteristics of the NanoString® cohort (n=63) .......... 10
   Table S7 Treatment outcomes in the NanoString® cohort (n=63) ................. 11
   Table S8 Differently expressed genes between responders and non-responders .... 12
   Table S9 Genes included in the NanoString® pathway analysis of B-cell receptor signaling .......... 13
   Table S10 Shrinkage of regression coefficients in the mRNA LASSO model ....... 13
4. Supplementary Figures
   Figure S1 Performance measures of prediction models ............................. 14-15
   Figure S2 Flowchart of included samples in NanoString® analysis .......... 16
   Figure S3 B-cell receptor signaling scores according to type of rejection ........ 17
   Figure S4 Correlation matrices of DE genes .................................................. 18
   Figures S5 Penalization and shrinkage in the mRNA.LASSO model ............. 19
5. Equation of ALEMAR-score ............................................................................. 20
6. References ....................................................................................................... 20
1. Detailed methods of gene expression profiling

Formalin-fixed paraffin-embedded biopsy samples on which the initial diagnosis of AR was made were obtained from the in-hospital pathology biobank. RNA was isolated as described previously. RNA concentration and quality were assessed using the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific Waltham, MA, USA). RNA samples with a concentration below 10ng/ul or a 260/280 ratio below 1.5 were excluded for further analysis.

The Banff Human Organ Transplant (B-HOT) panel was used in the NanoString® nCounter assay. The B-HOT panel was specifically designed for use in solid organ transplant research and consists of 758 target genes of interest and 12 reference genes for quality control and normalization. Capture and reporter probes of 770 target genes, 6 positive control probes and 8 negative control probes were added to 25 ng RNA of each sample for hybridization. Excess probes were removed by two times magnetic bead selection. Samples were loaded on nCounter Sample cartridges (NanoString® Technologies, Seattle, WA, USA) for alignment and immobilization. Next, cartridges were loaded onto the nCounter FLEX system (NanoString® Technologies) for imaging and counting of RNA-probe complexes. Excitation wavelength for fields of view (FOV) was set at 490 nm. Raw count complex (RCC) files were obtained and transferred to nSolver software for data analysis.

nSolver software (NanoString® Technologies) was used for quality control, normalization and data analysis. First, a simple analysis was performed for quality control (QC) of samples. Default parameters of QC flagging as recommended by the manufacturer were used. Each sample with a positive QC flag was evaluated and removed when needed. Next, the advanced analysis module was performed with all samples that passed the QC. For this analysis, a threshold was calculated to remove low count genes as recommended by the manufacturer as follows: The background for each sample was calculated as the average of negative controls plus 2 standard deviations (SD). The sample with maximum background was identified. The threshold of gene detection was calculated as 2 times the maximum background. All genes that had >50% of samples with a raw count below this threshold were omitted from the analysis. The geNorm algorithm was selected for the housekeeping (HK) gene normalization procedure. Samples that were far outlying other samples within this HK normalization were excluded from the analysis, i.e. samples with a normalization factor greater than -3 or +3 or a mean squared error (MSE) above 2.
2. TRIPOD checklist

| Section/Topic | Checklist Item                                                                 | Page |
|---------------|-------------------------------------------------------------------------------|------|
| Title and abstract |                                                                                   |      |
| Title         | Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted. | P1   |
| Abstract      | Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions. | P3   |
| Introduction  |                                                                                   |      |
| Background and objectives | Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models. | P4   |
|               | Specify the objectives, including whether the study describes the development or validation of the model or both. | P4   |
| Methods       |                                                                                   |      |
| Source of data | Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable. | P5   |
|               | Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up. | P5   |
| Participants  | Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres. | P5   |
|               | Describe eligibility criteria for participants. | P5   |
|               | Give details of treatments received, if relevant. | P5; Reference #7 |
| Outcome       | Clearly define the outcome that is predicted by the prediction model, including how and when assessed. | P7   |
|               | Report any actions to blind assessment of the outcome to be predicted. | -    |
| Predictors    | Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured. | P6; Table S1 |
|               | Report any actions to blind assessment of predictors for the outcome and other predictors. | -    |
| Sample size   | Explain how the study size was arrived at. | -    |
| Missing data  | Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method. | P8   |
| Statistical analysis methods | Describe how predictors were handled in the analyses. | P8; Table S1 |
|               | Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation. | P8-10 |
|               | Specify all measures used to assess model performance and, if relevant, to compare multiple models. | P9-10 |
| Risk groups   | Provide details on how risk groups were created, if done. | P9   |
| Results       |                                                                                   |      |
| Participants  | Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful. | P10; P13; Table 2; Table S7; Figure S2 |
|               | Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome. | P10; Table 1; Table S6 |
| Model development | Specify the number of participants and outcome events in each analysis. | P10; P13; Table 2; Table S7 |
|               | If done, report the unadjusted association between each candidate predictor and outcome. | -    |
| Model specification | Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point). | P11-12; P14; Figure 1; Table S2; Table S10 Figure S5 |
|               | Explain how to use the prediction model. | P12; Table 3; Supplementary file S |
| Model performance | 16 | Report performance measures (with CIs) for the prediction model. | P11; P14; Figure 4; Figure S1 |
|-------------------|----|---------------------------------------------------------------|-------------------------------|

**Discussion**

| Limitations | 18 | Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data). | P17 |
| Interpretation | 19b | Give an overall interpretation of the results, considering objectives, limitations, and results from similar studies, and other relevant evidence. | P15-17 |
| Implications | 20 | Discuss the potential clinical use of the model and implications for future research. | P15-18 |

**Other information**

| Supplementary information | 21 | Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets. | Throughout article |
| Funding | 22 | Give the source of funding and the role of the funders for the present study. | P2 |
Table S1: Collected baseline characteristics of the study population

| VARIABLES                                      | TYPE OF VARIABLE | USED IN PREDICTION MODEL | CODING IN PREDICTION MODEL |
|------------------------------------------------|------------------|---------------------------|---------------------------|
| **PATIENT CHARACTERISTICS**                   |                  |                           |                           |
| RECIPIENT AGE AT TRANSPLANTATION               | CONTINUOUS       | NO                        |                           |
| RECIPIENT AGE AT ACUTE REJECTION               | CONTINUOUS       | YES                       | YEARS, CONTINUOUS         |
| GENDER                                         | CATEGORICAL      | NO                        |                           |
| ETHNICITY                                      | CATEGORICAL      | NO                        |                           |
| PRIMARY KIDNEY DISEASE                        | CATEGORICAL      | NO                        |                           |
| TRANSPLANT NUMBER                              | CATEGORICAL      | NO                        |                           |
| PRE-EMPTIVE TRANSPLANTATION                    | CATEGORICAL      | YES                       | 0=NO; 1=YES               |
| % PANEL REACTIVE ANTIBODIES - CURRENT          | CONTINUOUS       | YES                       | PERCENTAGE, CONTINUOUS    |
| **TRANSPLANT CHARACTERISTICS**                |                  |                           |                           |
| TYPE OF DONOR (LIVING OR POSTMORTAL)           | CATEGORICAL      | YES                       | 1=LIVING; 2=POSTMORTAL    |
| DONOR AGE                                      | CONTINUOUS       | YES                       | YEARS, CONTINUOUS         |
| HLA MISMATCHES                                 | CATEGORICAL      | YES                       | 1-6 = NUMBER OF HLA MISMATCHES |
| HLA MISMATCHES DR                              | CATEGORICAL      | NO                        |                           |
| DELAYED GRAFT FUNCTION                         | CATEGORICAL      | YES                       | 0=NO; 1=YES               |
| **REJECTION CHARACTERISTICS**                 |                  |                           |                           |
| TIMING OF REJECTION (DAYS AFTER TRANSPLANTATION)| CONTINUOUS       | YES                       | DAYS, CONTINUOUS          |
| EARLY VS LATE*                                 | CATEGORICAL      | NO                        |                           |
| TYPE OF REJECTION (HISTOLOGICAL CLASSIFICATION)| CATEGORICAL      | YES                       | TYPE_REJECTION_2=AABMR (REFERENCE ATCMR) |
|                                                  |                  |                           | TYPE_REJECTION_3=MIXED-TYPE (REFERENCE ATCMR) |
| DSA                                           | CATEGORICAL      | YES                       | 0= NO DSA; 1=DSA          |
| MAX BASELINE EGFR**                            | CONTINUOUS       | YES                       | EGFR, CONTINUOUS          |
| **THERAPY CHARACTERISTICS**                   |                  |                           |                           |
| TRIPLE MAINTENANCE THERAPY (TAC+MMF+PRED)**    | CATEGORICAL      | YES                       | 0=NO; 1=YES               |
| DOSAGE FREQUENCY OF ALEMTUZUMAB                | CATEGORICAL      | YES                       | 0= 1 DOSAGE OF 30MG; 1= 2 DOSAGES OF 30MG |
| INDICATION FOR ALEMTUZUMAB (SEVERE OR GLUCOCORTICOID-RESISTANT AR) | CATEGORICAL      | YES                       | 1=SEVERE AR; 2=GLUCOCORTICOID-RESISTANT AR |

*early rejection<3 months after transplantation, late rejection>3 months after transplantation

**Max Baseline eGFR= highest eGFR in the 3 months prior to alemtuzumab

***TAC=tacrolimus, MMF=mycophenolate mofetil, PRED=methylprednisolone
Table S2: Shrinkage of regression coefficients in the lambda.min LASSO model

| COVARIATES                        | COEFFICIENTS BEFORE SHRINKAGE | COEFFICIENTS AFTER SHRINKAGE |
|-----------------------------------|------------------------------|-------------------------------|
| RECIPIENT AGE AT AR               | -0.038                       | -0.005                        |
| PRE-EMPTIVE TRANSPLANTATION       | -0.915                       | 0.000                         |
| PRA CURRENT                       | -0.075                       | -0.008                        |
| DONOR AGE                         | -0.045                       | -0.000                        |
| TYPE OF DONOR                     | -1.141                       | 0.000                         |
| HLA MISMATCHES                    | -0.847                       | -0.371                        |
| TRIPLE MAINTENANCE THERAPY        | -1.588                       | -0.775                        |
| ALEM DOSES*                       | -0.545                       | -0.066                        |
| INDICATION FOR ALEMTUZUMAB        | 1.349                        | 0.138                         |
| TIMING OF REJECTION               | 0.001                        | 0.001                         |
| TYPE OF REJECTION: AABMR          | -0.761                       | -0.215                        |
| TYPE OF REJECTION: MIXED-TYPE     | 1.741                        | 0.432                         |
| DELAYED GRAFT FUNCTION            | -0.824                       | 0.000                         |
| DSA                               | -0.032                       | 0.000                         |
| MAX BASELINE EGFR                 | -0.057                       | -0.004                        |

* Alem doses = Dosage frequency of alemtuzumab
Table S3: Shrinkage of regression coefficients in the lambda.1SE LASSO model

| COVARIATES                        | COEFFICIENTS BEFORE SHRINKAGE | COEFFICIENTS AFTER SHRINKAGE |
|-----------------------------------|------------------------------|------------------------------|
| RECIPIENT AGE AT AR               | -0.038                       | 0.00000                      |
| PRE-EMPTIVE TRANSPLANTATION       | -0.915                       | 0.00000                      |
| PRA CURRENT                       | -0.075                       | 0.00000                      |
| DONOR AGE                         | -0.045                       | 0.00000                      |
| TYPE OF DONOR                     | -1.141                       | 0.00000                      |
| HLA MISMATCHES                    | -0.847                       | -0.09575                     |
| TRIPLE MAINTENANCE THERAPY        | -1.588                       | -0.29382                     |
| ALEM DOSES*                       | -0.545                       | 0.00000                      |
| INDICATION FOR ALEMTUZUMAB        | 1.349                        | 0.00000                      |
| TIMING OF REJECTION               | 0.001                        | 0.00021                      |
| TYPE OF REJECTION: AABMR          | -0.761                       | 0.00000                      |
| TYPE OF REJECTION: MIXED-TYPE     | 1.741                        | 0.00000                      |
| DELAYED GRAFT FUNCTION            | -0.824                       | 0.00000                      |
| DSA                               | -0.032                       | 0.00000                      |
| MAX BASELINE EGFR                 | -0.057                       | 0.00000                      |

* Alem doses = Dosage frequency of alemtuzumab
Table S4: Bootstrap variable selection

| VARIABLES                                      | % SELECTED IN RESAMPLING | % SELECTED IN RESAMPLING |
|------------------------------------------------|---------------------------|---------------------------|
|                                                 | – CONSTANT $\lambda^{**}$ | – VARIABLE $\lambda^{**}$ |
| (INTERCEPT)                                     | 100.0                     | 100.0                     |
| RECIPIENT AGE AT AR                             | 59.2                      | 78.9                      |
| PRE-EMPTIVE TRANSPLANTATION                    | 11.7                      | 49.9                      |
| PRA CURRENT                                    | 69.5                      | 88.4                      |
| DONOR AGE                                      | 38.0                      | 72.1                      |
| TYPE OF DONOR                                   | 25.9                      | 57.9                      |
| HLA MISMATCHES                                 | 98.4                      | 99.6                      |
| TRIPLE MAINTENANCE THERAPY                     | 85.9                      | 91.1                      |
| ALEM DOSES                                     | 49.3                      | 69.0                      |
| INDICATION FOR ALEMTUZUMAB                     | 53.8                      | 79.9                      |
| TIMING OF REJECTION                             | 82.8                      | 88.4                      |
| TYPE OF REJECTION: AABMR                       | 59.9                      | 71.4                      |
| TYPE OF REJECTION: MIXED-TYPE                  | 67.8                      | 83.0                      |
| DELAYED GRAFT FUNCTION                         | 4.4                       | 31.3                      |
| DSA                                            | 18.3                      | 46.7                      |
| MAX BASELINE EGFR                              | 62.2                      | 87.9                      |

* Alem doses = Dosage frequency of alemtuzumab

** Two internal validations using bootstrap resampling have been performed; first to evaluate the robustness of the model (constant $\lambda$) and second to evaluate the tuning parameter (variable $\lambda$)
Table S5: Logistic regression model: Included variables and statistics

| VARIABLES                          | OR  | 95% CI       | P-VALUE |
|------------------------------------|-----|--------------|---------|
| (INTERCEPT)                        | 26.896 | 4.274 – 222.620 | 0.001   |
| HLA MISMATCHES                     | 0.519 | 0.342 – 0.749 | 0.001   |
| TRIPLE MAINTENANCE THERAPY         | 0.091 | 0.025 – 0.282 | 0.000   |
| MAX BASELINE EGFR                  | 0.973 | 0.947 – 0.997 | 0.037   |
Table S6: Baseline characteristics of the NanoString® cohort (n=63)

| VARIABLES                              | MISSING (N)* | ALL PATIENTS (N=63) | RESPONDERS (N=46) | NON-RESPONDERS (N=15) | P-VALUE |
|----------------------------------------|--------------|---------------------|------------------|-----------------------|---------|
| **PATIENT CHARACTERISTICS**            |              |                     |                  |                       |         |
| Recipient age at transplantation (YEARS), median (IQR**) | 57.1 (43.0-63.4) | 56.8 (41.9-63.4) | 55.0 (42.9-63.4) | 0.940 |
| Recipient age at acute rejection (YEARS), median (IQR) | 57.1 (43.1-63.9) | 56.8 (41.9-64.0) | 55.5 (44.8-63.4) | 0.927 |
| Gender (male), N (%)                    | 39 (62%)     | 28 (61%)            | 10 (67%)         | 0.687                 |
| Ethnicity (Caucasian), N (%)            | 39 (62%)     | 28 (61%)            | 9 (60%)          | 0.684                 |
| Primary kidney disease, N (%)           |              |                     |                  |                       |         |
| Hypertension                           | 11 (17%)     | 6 (13%)             | 3 (20%)          | 0.817                 |
| Diabetic nephropathy                   | 15 (24%)     | 12 (26%)            | 3 (20%)          |                       |
| Glomerulonephritis                     | 5 (8%)       | 3 (7%)              | 2 (13%)          |                       |
| Polycystic kidney disease              | 11 (17%)     | 8 (17%)             | 3 (20%)          |                       |
| Reflux nephropathy                     | 5 (8%)       | 5 (11%)             | 0 (0%)           |                       |
| Other                                  | 15 (24%)     | 11 (24%)            | 4 (27%)          |                       |
| Unknown                                | 1 (2%)       | 1 (2%)              | 0 (0%)           |                       |
| Transplant number (first), N (%)       | 52 (83%)     | 38 (83%)            | 13 (87%)         | 1.000                 |
| Pre-emptive transplantation, N (%)     | 19 (30%)     | 12 (26%)            | 6 (40%)          | 0.340                 |
| % Panel reactive antibodies - current, median (IQR) | 0.0 (0.0-4.0) | 0.0 (0.0-4.0) | 0.0 (0.0-4.0) | 0.555 |
| **TRANSPLANT CHARACTERISTICS**         |              |                     |                  |                       |         |
| Type of donor (Living), N (%)          | 41 (65%)     | 31 (67%)            | 9 (60%)          | 0.601                 |
| Donor age (years), median (IQR)        | 54.0 (43.5-64.0) | 55.0 (46.0-64.0) | 53.0 (38.5-60.0) | 0.244 |
| HLA Mismatches, median (IQR)           | 4.0 (2.25-5.0) | 4.0 (3.0-5.0) | 3.0 (2.0-4.5) | 0.087 |
| HLA Mismatches DR, N (%)               | 1            |                     |                  |                       | 0.780   |
| 0                                      | 9 (14%)      | 6 (13%)             | 3 (20%)          |                       |
| 1                                      | 30 (48%)     | 23 (51%)            | 6 (40%)          |                       |
| 2                                      | 23 (37%)     | 16 (36%)            | 6 (40%)          |                       |
| Delayed graft function, N (%)          | 25 (40%)     | 21 (46%)            | 3 (20%)          | 0.077                 |
| **REJECTION CHARACTERISTICS**          |              |                     |                  |                       |         |
| Timing of rejection (days after \(T\)) | 11.0 (7.0-324.0) | 9.5 (6.0-94.3) | 461.0 (201.0-971.0) | 0.000 |
| Early***, N (%)                        | 39 (62%)     | 34 (74%)            | 3 (20%)          | 0.000                 |
| Histological rejection category, N (%) |              |                     |                  |                       | 0.188   |
| ATCMR                                  | 44 (70%)     | 33 (72%)            | 10 (67%)         |                       |
| AABMR                                  | 11 (17%)     | 9 (20%)             | 1 (7%)           |                       |
| Mixed                                  | 8 (13%)      | 4 (9%)              | 4 (27%)          |                       |
| DSA, N (%)                             | 13 (21%)     | 9 (20%)             | 4 (27%)          | 0.718                 |
| Max baseline eGFR***, median (IQR)     | 26.0 (2.5-46.8) | 26.0 (1.25-45.0) | 26.0 (7.5-48.3) | 0.953 |
| **THERAPY CHARACTERISTICS**            |              |                     |                  |                       |         |
| Triple maintenance therapy (TAC+MMF+PRED)*****, N (%) | 44 (70%) | 35 (76%) | 7 (47%) | 0.053 |
| Dosage frequency of alemtuzumab (single), N (%) | 59 (94%) | 43 (94%) | 14 (93%) | 1.000 |
| Indication for alemtuzumab (Severe AR), N (%) | 17 (27%) | 14 (30%) | 2 (13%) | 0.312 |

*The patient with missing data was excluded in all prediction models **IQR = interquartile range ***early rejection<3 months after transplantation, late rejection>3 months after transplantation ****Max Baseline eGFR= highest eGFR in the 3 months prior to
**alemtuzumab**

*Tac=tacrolimus, MMF=mycophenolate mofetil, PRED=methylprednisolone; aTCMR, acute T cell-mediated rejection; aABMR, acute antibody-mediated rejection*

### Table S7: Treatment outcomes in the NanoString® cohort (n=63)

| VARIABLES                                | VALUE            |
|------------------------------------------|------------------|
| EVENTS                                   |                  |
| DEATH WITH FUNCTIONING GRAFT*, N (%)     | 2 (3%)           |
| TIME INTERVAL (DAYS)**, MEAN ±SD         | 103 ±42.4        |
| ALLOGRAFT LOSS, N (%)                    | 8 (13%)          |
| TIME INTERVAL (DAYS), MEDIAN (IQR****)   | 93.0 (93.0-95.8) |
| LOST TO FOLLOW UP, N (%)                 | 0 (0%)           |
| EGFR                                     |                  |
| NUMBER OF MEASUREMENTS***, MEDIAN (IQR)  | 26.0 (18.5-33.0) |
| 6 MONTHS AFTER ALEMTUZUMAB, MEDIAN (IQR) | 36.0 (28.5-42.9) |
| RESPONSE TO ALEMTUZUMAB                  |                  |
| RESPONDERS, N (%)                        | 46 (73%)         |
| NON-RESPONDERS, N (%)                    | 15 (24%)         |

*Causes of death: cardiac arrest during pneumonia and cardiac decompensation (day 73 after alemtuzumab); pneumosepsis (day 133 after alemtuzumab)

** Days after alemtuzumab treatment

*** IQR= Interquartile range

**** Number of eGFR measurements during the follow-up period
Table S8: Differently expressed genes between responders and non-responders

| GENE       | LOG2 FOLD CHANGE | STANDARD ERROR | P-VALUE     | BH. P-VALUE* |
|------------|------------------|----------------|-------------|--------------|
| XBP1       | 1.2              | 0.178          | 7.74E-09    | 4.07E-06     |
| IGLC1      | 3.19             | 0.591          | 1.23E-06    | 0.000324     |
| IGHM       | 2.59             | 0.582          | 3.97E-05    | 0.00525      |
| IGHG3      | 3.61             | 0.831          | 5.67E-05    | 0.00525      |
| PRDM1      | 1.3              | 0.3            | 5.87E-05    | 0.00525      |
| IGHG1      | 3.78             | 0.881          | 6.61E-05    | 0.00525      |
| IGHG2      | 3.6              | 0.841          | 6.99E-05    | 0.00525      |
| IGHG4      | 3.66             | 0.867          | 8.59E-05    | 0.00565      |
| TPSAB1/B2  | 1.81             | 0.444          | 0.000136    | 0.00793      |
| IGKC       | 3.13             | 0.772          | 0.000151    | 0.00793      |
| CXCR4      | 1.04             | 0.32           | 0.0019      | 0.0771       |
| S100A8     | -1.5             | 0.466          | 0.00207     | 0.0778       |
| CD209      | 1.01             | 0.322          | 0.00256     | 0.0898       |

*BH. p-value= Benjamin-Hochberg corrected p-value
Table S9: Genes included in the NanoString® pathway analysis of B-cell receptor signaling

| B-CELL RECEPTOR SIGNALING GENES |
|----------------------------------|
| BLK    | IGHG1 | NFKBIA |
| BLNK   | IGHG2 | NPHS1  |
| BTK    | IGHG3 | PIK3CD |
| CD19   | IGHG4 | PPP3CA |
| CD22   | IGHM  | PSMB10 |
| CD72   | IGKC  | PSMB8  |
| CD79A  | IGLC1 | PSMB9  |
| CD81   | IKBKB | PSME1  |
| CHUK   | IKBKG | PSME2  |
| FCGR2B | INPP5D| PTPN6  |
| FKBP1A | JUN   | RAF1   |
| FOS    | MAPK3 | REL    |
| FYN    | NFATC1| RELA   |
| IFITM1 | NFATC2| SLAMF8 |
| IGHA1  | NFKB1 | SYK    |

Table S10: Shrinkage of regression coefficients in the mRNA LASSO model

| COVARIATES  | COEFFICIENTS BEFORE SHRINKAGE | COEFFICIENTS AFTER SHRINKAGE |
|-------------|-------------------------------|-----------------------------|
| ALEMAR-SCORE | 8.06610                       | 7.552635                    |
| XBP1        | 0.00123                       | 0.001154                    |
| IGHG1       | 0.00004                       | 0.000001                    |
| CXCR4       | 0.00091                       | 0.000591                    |
| S100A8      | -0.00242                      | -0.001069                   |
Figure S1: Performance measures of prediction models

Performance was compared between LASSO models and the logistic regression model by evaluation of the discrimination and calibration plots.
(a-b) Discrimination of the lambda.min model was good ($c$-index=0.858) and the calibration showed a good agreement (slope=1.955)

(c-d) Discrimination and calibration of the lambda.1SE model was inferior to the lambda.min model ($c$-index=0.808, slope=5.113)

(e-f) Discrimination of the logistic regression model was lower compared to the lambda.min model ($c$-index=0.814), while the calibration was slightly better (slope=1.000)

*$AUC=area \text{ under the curve (equal to } c\text{-index); Black dots in calibration plots represent the sample percentiles).*
FFPE tissues were available in 91 of 115 patients of the study cohort. Examination of RNA concentration and quality resulted in exclusion of 19 samples. Another 10 samples were excluded by the multistep normalization within the nSolver software, including quality control flags, positive and negative controls and housekeeping genes. Sixty-three samples could be used in the data analysis.

Figure S2: Flowchart of included samples in NanoString® analysis
Figure S3: B-cell receptor signaling scores according type of rejection.
NanoString® pathway analysis for B-cell receptor signaling (BCR) related genes calculates a score for each patient based on the overall expression of BCR genes. No association was found between the BCR score and the type of rejection (aTCMR versus aABMR/mixed-type).
Figure S4: Correlation matrices of DE genes

(a-b) Correlation matrices including all DE genes that were considered for inclusion in the prediction model. On the diagonal are the names of the mRNA markers. The lower triangle shows the scatterplot between two markers including a smoothing line. The upper triangle shows the Spearman correlation coefficient. Correlations varied between 0.066 and 0.99.

(c) Correlation matrix of the DE genes that were selected for inclusion shows a maximum correlation of 0.54.
Figure S5: Penalization and shrinkage in the mRNA LASSO model
LASSO method was used for shrinkage and selection of variables to include in the mRNA prediction model. (a) The tuning parameter (λ) corresponding to the minimal cross validated error was used conform the main model (left dotted vertical line). (b) The shrinkage factor (s=0.13) corresponding to the minimal lambda didn’t shrink any of the 5 variables to zero. Positive variables (red) give a higher risk of non-response to alemtuzumab, while negative variables (green) give a lower risk of non-response.
5. Equation of ALEMAR-score

\[ \ln\left( \frac{p}{1-p} \right) = 0.59118 - 0.00489 \times (\text{Recipient age at AR}) - 0.00759 \times (\text{PRA current}) - 0.3713 \times (\text{HLA mismatches}) - 0.7747 \times (\text{Triple maintenance therapy}) - 0.06608 \times (\text{Alcm doses}) + 0.13827 \times (\text{Indication for alemtuzumab}) + 0.0058 \times (\text{Timing of rejection}) - 0.21507 \times (\text{Type of rejection: aABMR}) + 0.43233 \times (\text{Type of rejection: MIXED-type}) - 0.00441 \times (\text{Max baseline eGFR}) \]

6. References

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