Response

KIR/HLA incompatibility in HIV-1 transmission and importance of the ‘missing self’ model

We appreciate the interest of Drs Behrendt and Zaia in our recent study of allogeneic killer cell immunoglobulin-like receptor (KIR)/HLA combinations in HIV-1 transmission,1 and we are grateful for the opportunity to respond to their comments. Behrendt and Zaia reanalyzed our data by testing regrouped “missing ligand” combinations consisting of recipient partner KIRs lacking cognate index partner HLAs, reporting that they are not associated with HIV-1 transmission. In our analysis, however, we specifically studied “missing self” combinations, which also take recipient HLA into account, adding the requirement that natural killer (NK) cells have to be educated by self HLA before they can sense the lack of it on incoming target cells.2 Missing self, but not missing ligand, combinations were shown to be beneficial during haploidentical hematopoietic stem cell transplantation as a treatment of acute myeloid leukemia, inducing alloreactive NK cells that help prevent leukemia relapse and graft-versus-host disease.3,4 Conversely, the “matched” combinations we tested consisted of recipient KIRs recognizing index HLAs regardless of recipient HLA, because recipient NK cells will be inhibited by index HLA regardless of whether they have been educated or not. Our analyses (see Table 2 in Jennes et al1) showed that HIV-1 transmission and lack thereof correlated with 1 such matched combination (recipient KIR2DL3/KIR2DL1 with index HLA-C1/C2) and 1 such missing self combination (recipient KIR2DL1 with HLA-C1/C2 and index HLA-C1/C1), respectively, both of which are diluted out in the regrouped analyses by Behrendt and Zaia.

For reasons of simplicity, we chose to analyze all relevant KIR2DL1/HLA-C matched and missing self subset combinations in a single step. Although recipient KIR2DL3/KIR2DL3 with index HLA-C1 and recipient KIR2DL1/HLA-C2 with index HLA-C1/C1 also yielded statistically or near-statistically significant differences (P values of .019 and .075, respectively, not shown in our paper), our subset analyses immediately showed that these differences were only valid for index HLA-C1/C2 and recipient HLA-C1/C2, respectively (P values of .001 and .023, respectively, as shown in our paper). However, we agree that it is not clear why the alternative missing self combinations for recipient KIR2DL2 or KIR2DL3 in our study was a hypothesis generating exploratory analysis of observational data and because testing was limited to a number of preplanned and scientifically plausible comparisons.5,6 In the analysis concerned, we performed 16 such comparisons and not 36 as Behrendt and Zaia state we had sufficient power to detect the identified matched and missing self combinations at P < .05. It is unclear to us on what basis they state we had sufficient power to perform only 1 comparison. Even if a standard Bonferroni correction would have been applied, which is far too conservative given independence resulting from KIR linkage and allelic distribution of KIR2DL2/KIR2DL3 and HLA-C1/C2, the matched combination would still remain statistically significant. Our study is the first to suggest a role for allogeneic KIR/HLA interactions and alloreactive NK cells in protection against HIV-1 transmission. Because of the novelty of our findings, the relatively small size of our cohort, and that we did not correct for multiple testing, independent confirmation of our findings in other cohorts is certainly warranted.

References


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Molecular classification of mature aggressive B-cell lymphoma using digital multiplexed gene expression on formalin-fixed paraffin-embedded biopsy specimens

The most frequent mature aggressive B-cell lymphomas are diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). Patients suffering from molecularly defined BL (mBL) but treated with a regimen developed for DLBCL show an unfavorable outcome compared with mBL treated with chemotherapy regimens for BL.1 Distinguishing BL from DLBCL by conventional histopathology is challenging in lymphomas that have features common to both diseases (aggressive B-cell lymphoma unclassifiable with features of DLBCL and BL [intermediates]).2 Moreover, DLBCLs are a heterogeneous group of lymphomas comprising distinct molecular subtypes: the activated B-cell–like (ABC), the germinal center B-cell–like (GCB), and the unclassifiable subtype as defined by gene expression profiling (GEP).3 Attempts to replace GEP with techniques applicable to formalin-fixed paraffin-embedded (FFPE) tissue led to algorithms for immunohistochemical staining (IHS).4 Disappointingly, the algorithms for identifying mBL among mature aggressive B-cell lymphomas were analyzed using DMGE (NanoString Technologies, Seattle, WA; see supplemental Materials for detailed methods) of FFPE- and fresh-frozen tissue blocks (Figure 1B). All discrepancies were classified as mBL (both lymphomas carried a t(8;14) translocation). No major mistake (a classification of a non-mBL as mBL or vice versa) was observed. This performance is comparable with that of DMGE-based predictions with the gold standard (array) (Figure 1A). The 2 divergent classifications were 1 case of mBL that was classified as intermediate and 1 case of an intermediate that was classified as mBL [both lymphomas carried a t(8;14) translocation], No major mistake (a classification of a non-mBL as mBL or vice versa) was observed. This performance is comparable with that of DMGE data from fresh-frozen tissue blocks (supplemental Materials). Compared with the array, 5 of 31 (16%) non-mBL/DLBCLs received different molecular classifications using DMGE and FFPE-derived RNA (Figure 1B). All discrepancies comprised lymphomas that switched between the unclassified and ABC or unclassified and GCB labels. Again, no major mistake (a classification of a GCB as an ABC or vice versa) was observed. In contrast, the Hans IHS algorithm7 led to major mistakes (2 GCB classified as non-GCB and 1 ABC as GCB; Figure 1B).

For FFPE-derived RNA, the classification of only 2 of 39 samples (5%) differed, when comparing DMGE-based predictions with the gold standard (array) (Figure 1A). The 2 divergent classifications were 1 case of mBL that was classified as intermediate and 1 case of an intermediate that was classified as mBL [both lymphomas carried a t(8;14) translocation]. No major mistake (a classification of a non-mBL as mBL or vice versa) was observed. This performance is comparable with that of DMGE data from fresh-frozen tissue blocks (supplemental Materials). Compared with the array, 5 of 31 (16%) non-mBL/DLBCLs received different molecular classifications using DMGE and FFPE-derived RNA (Figure 1B). All discrepancies comprised lymphomas that switched between the unclassified and ABC or unclassified and GCB labels. Again, no major mistake (a classification of a GCB as an ABC or vice versa) was observed. In contrast, the Hans IHS algorithm7 led to major mistakes (2 GCB classified as non-GCB and 1 ABC as GCB; Figure 1B).

GEP-based molecular classification of mature aggressive B-cell lymphomas is possible with FFPE-derived RNA at a reasonable cost (<100 Euro) and within a reasonable period of time (24 hours). To the best of our knowledge, this is the first molecular method for identifying mBL among mature aggressive B-cell lymphomas using FFPE-derived RNA.

To the editor:

Molecular classification of mature aggressive B-cell lymphoma using digital multiplexed gene expression on formalin-fixed paraffin-embedded biopsy specimens

The most frequent mature aggressive B-cell lymphomas are diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). Patients suffering from molecularly defined BL (mBL) but treated with a regimen developed for DLBCL show an unfavorable outcome compared with mBL treated with chemotherapy regimens for BL.1 Distinguishing BL from DLBCL by conventional histopathology is challenging in lymphomas that have features common to both diseases (aggressive B-cell lymphoma unclassifiable with features of DLBCL and BL [intermediates]).2 Moreover, DLBCLs are a heterogeneous group of lymphomas comprising distinct molecular subtypes: the activated B-cell–like (ABC), the germinal center B-cell–like (GCB), and the unclassifiable subtype as defined by gene expression profiling (GEP).3 Attempts to replace GEP with techniques applicable to formalin-fixed paraffin-embedded (FFPE) tissue led to algorithms for immunohistochemical staining (IHS).4 Disappointingly, the algorithms yielded conflicting results with respect to their prognostic potential, raising concerns about their validity.5 Furthermore, IHS algorithms did not provide a fully resolved classification: they did not identify mBL nor did they separate ABC from unclassified DLBCLs.4

We used digital multiplexed gene expression (DMGE) with FFPE-derived RNA to classify aggressive B-cell lymphomas. Our assay comprised only 30 genes (10 for the detection of mBL and 20 for the detection of ABC and GCB). We chose these genes by reanalysis of the microarray data reported in a previous study.6 A detailed description of the methods is provided in the supplemental Materials on the Blood website. Thirty-nine samples from mature aggressive B-cell lymphomas were analyzed using DMGE (nCounter; NanoString Technologies, Seattle, WA; see supplemental Materials for detailed methods) of FFPE- and fresh-frozen RNA. All cases were previously characterized by the Molecular Mechanisms of Malignant Lymphoma6 consortium using the Affymetrix GeneChip technology (gold standard of classification).

For FFPE-derived RNA, the classification of only 2 of 39 samples (5%) differed, when comparing DMGE-based predictions with the gold standard (array) (Figure 1A). The 2 divergent classifications were 1 case of mBL that was classified as intermediate and 1 case of an intermediate that was classified as mBL [both lymphomas carried a t(8;14) translocation]. No major mistake (a classification of a non-mBL as mBL or vice versa) was observed. This performance is comparable with that of DMGE data from fresh-frozen tissue blocks (supplemental Materials). Compared with the array, 5 of 31 (16%) non-mBL/DLBCLs received different molecular classifications using DMGE and FFPE-derived RNA (Figure 1B). All discrepancies comprised lymphomas that switched between the unclassified and ABC or unclassified and GCB labels. Again, no major mistake (a classification of a GCB as an ABC or vice versa) was observed. In contrast, the Hans IHS algorithm7 led to major mistakes (2 GCB classified as non-GCB and 1 ABC as GCB; Figure 1B).

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