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BRIEF REPORT

Allelic variation in *HLA-DRB1* is associated with development of antidrug antibodies in cancer patients treated with atezolizumab that are neutralizing in vitro

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Abstract

The treatment of diseases with biologic agents can result in the formation of antidrug antibodies (ADA). Although drivers for ADA formation are unknown, a role for antigen presentation is likely, and variation in human leukocyte antigen (HLA) genes has been shown to be associated with occurrence of ADA for several biologics. Here, we performed an HLA-wide association study in 1982 patients treated with the anti-PD-L1 antibody atezolizumab across eight clinical trials. On average, 29.8% of patients were ADA-positive ($N = 591$, range of 13.5%–38.4% per study) and 14.6% of patients were positive for ADA that were neutralizing in vitro (neutralizing antibodies [NAb], $N = 278$, range of 6.4%–21.9% per study). In a meta-analysis of logistic regression coefficients, we found statistically significant associations between HLA class II alleles and ADA status. The top-associated alleles were *HLA-DRB1*01:01* in a comparison of ADA-positive versus ADA-negative patients ($p = 3.4 \times 10^{-5}$, odds ratio [OR] 1.96, 95% confidence interval [95% CI] 1.64–2.28) and *HLA-DQA1*01:01* when comparing NAb-positive with ADA-negative patients ($p = 2.8 \times 10^{-7}$, OR 2.31, 95% CI 1.98–2.66). Both alleles occur together on a common HLA haplotype, and analyses considering only NAb-negative, ADA-positive patients did not yield significant results, suggesting that the genetic association is mainly driven by NAb status. In conclusion, our study showed that HLA class II genotype is associated with the risk of developing ADA, and specifically NAb, in patients treated with atezolizumab, but the effect estimates suggest that immunogenetic factors are not sufficient as clinically meaningful predictors.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Antidrug antibodies (ADA) can impact the efficacy of therapeutics, but the mechanisms underlying their formation are poorly understood. Allelic variation

in human leukocyte antigen (HLA) proteins has been found to be associated with ADA against several biologics.

WHAT QUESTION DID THIS STUDY ADDRESS?

Is there an association between HLA genetic variation and the risk for development of ADA or neutralizing antibodies (NAb)?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

In 1982 patients across eight atezolizumab clinical trials we showed that a common haplotype of HLA class II alleles is associated with ADA and NAb risk. The association was consistent across cancer indications. Further research will be needed to uncover potential further associations in non-European patient populations.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The impact of HLA genetic variation on atezolizumab ADA development is not expected to be clinically relevant; however, it contributes to our understanding of the complex pattern of patient factors underlying ADA formation and might help in developing multifactorial prediction methods.

INTRODUCTION

Immune checkpoint blockade (ICB) using therapeutic antibodies has significantly improved clinical outcome and quality of life across a range of cancer indications.[1] However, the administration of therapeutic proteins (biologics) can lead to unwanted immune responses in the form of antidrug antibodies (ADA),[2] which have indeed been observed for all approved immune checkpoint inhibitors (ICI).[3,4] Reported ADA incidences for atezolizumab, a monoclonal antibody used as immunotherapeutic treatment in cancer patients, are higher than those for other anti PD1/PD-L1 antibodies when they are used as single-agent therapy.[3,4] A subset of ADA-positive patients develop neutralizing antibodies (NAb). By definition, NAb can inhibit the function of a protein therapeutic in vitro regardless of their in vivo clinical relevance.[2] Two recently published articles evaluated the impact of atezolizumab ADA and NAb on pharmacokinetics and clinical efficacy.[5,6] A trend towards lower atezolizumab exposure was observed in patients with ADA and NAb, but patients had sufficient exposure regardless of ADA status. Meta-analysis in over 10 trials showed that in spite of numerical differences in overall and progression-free survival in some studies, ADA-positive patients from studies with an overall treatment effect did benefit from atezolizumab.

The role of patient-related baseline prognostic factors in mediating the risk for ADA formation, and the need to take these into account when assessing potential ADA impact, is under active investigation.[7–9] Given their role in the presentation of peptide antigens to T cells, it seems likely that inherited genetic variation in human leukocyte antigen (HLA) molecules would play a role in ADA development. HLA proteins show a high degree of allelic variation, and the amino acid composition of their antigen-binding groove determines the spectrum of peptides presented.[10] Indeed, the risk of ADA development during treatment with therapeutic proteins such as interferon beta (IFNβ) or with antitumor necrosis factor (anti-TNF) antibodies was previously reported to be associated with specific HLA class II alleles.[11–13] We therefore hypothesized that the variable presentation of atezolizumab peptides via HLA molecules could contribute to ADA formation. Here, we present what is, to our knowledge, the largest genetic association study for an immunogenicity phenotype, involving a total of 1982 cancer patients treated with atezolizumab. We found statistically significant associations of HLA class II alleles with both ADA and NAb status, and fine-mapped both associations to a single amino acid residue in the HLA-DRß1 subunit.

METHODS

Studies and subjects

Patient data from eight atezolizumab phase III clinical trials were included in this analysis (Table 1). Clinical trial results have been previously reported,[14–21] and the clinical trial protocols have been provided as supplementary materials in the original study publications. Patients included in this study signed an optional Research Biosample Repository (RBR) Informed Consent Form (ICF) and provided whole blood samples. By signing, patients provided informed consent for analysis of inherited and non-inherited genetic variation from whole blood samples. Ethics Committees (EC) and Institutional Review Boards (IRB) in each country

TABLE 1 Number of patients with available human leukocyte antigen allele data, as well as antidrug antibodies and neutralizing antibody frequencies

Study name (study ID)	Indication	Treatment	ADA results *n*	ADA+ *n* (%)	NAb results *n*	NAb+ *n* (%)
IMvigor211 (GO29436)	Urothelial cancer	Atezolizumab	216	78 (36.1%)	208	40 (19.2%)
IMmotion151 (WO29637)	Renal cancer	Atezolizumab + Bev	243	57 (23.5%)	242	47 (19.4%)
IMpassion130 (WO29522)	TNBC	Atezolizumab + NAb-paclitaxel	237	32 (13.5%)	233	15 (6.4%)
IMpower130 (GO29537)	NSCLC	Atezolizumab + NAb-paclitaxel + CarboP	215	45 (20.9%)	208	16 (7.7%)
IMpower131 (GO29437)	NSCLC	All atezolizumab treated	360	132 (36.7%)	357	54 (15.1%)
		Atezolizumab + NAb-paclitaxel + CarboP	185	44 (23.8%)	184	14 (8.2%)
		Atezolizumab + paclitaxel + CarboP	175	88 (50.3%)	173	40 (23.1%)
IMpower132 (GO29438)	NSCLC	All atezolizumab treated	138	49 (35.5%)	137	30 (21.9%)
		Atezolizumab + pemetrexed + CisP	48	16 (33.3%)	47	12 (25.5%)
		Atezolizumab + pemetrexed + CarboP	90	33 (36.7%)	90	18 (20.0%)
IMpower150 (GO29436)	NSCLC	All atezolizumab treated	481	185 (38.5%)	436	76 (17.4%)
		Atezolizumab + Bev + paclitaxel + CarboP	229	89 (38.9%)	207	36 (17.4%)
		Atezolizumab + paclitaxel + CarboP	252	96 (38.1%)	229	40 (17.5%)
IMpower133 (GO30081)	SCLC	Atezolizumab + CarboP + etoposide	92	13 (14.1%)	91	0 (0%)
All		All atezolizumab treated	1982	591 (29.8%)	1912	278 (14.5%)

Abbreviations: ADA, antidrug antibodies; Bev, bevacizumab; CarboP, carboplatin; CisP, cisplatin; NAb, neutralizing antibodies; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; TNBC, triple-negative breast cancer.

and each study site for each clinical trial approved the clinical trial protocol, the main study ICF, and the RBR ICF. All EC and IRB forms are provided as Appendix S3. Some of the trials investigated in recent analyses of atezolizumab immunogenicity were not considered in the present study due to a lack of consent language for exploratory genetic analyses in the trial protocols.[5,6]

Detection and analysis of ADA and NAb status

ADA and NAb incidences were systematically and consistently assessed in atezolizumab-treated patients in all studies. Analytical methods were developed and run in accordance with industry best practices and health authority guidelines, and are described in more detail in Appendix S1.[2,22–26] Based on a tiered testing strategy, all ADA samples were tested in an ADA screening assay.[27] Samples that were deemed ADA-positive were subsequently analyzed in a NAb assay. The NAb assay utilized a ligand-binding format, and was designed in accordance with industry best practices.[28] Samples were pretreated prior to NAb analysis to separate ADA from drug in the sample. The resulting ADA solution was then analyzed for its ability to prevent the binding of drug to the PD-L1 target. Based on this, ADA-positive samples were deemed Nab-positive, Nab-negative, or Nab-indeterminate. As with all NAb assays, ADA/drug stoichiometry does not reflect in vivo conditions, and consequently samples that are found to be neutralizing in vitro may not impact drug efficacy.[2]

Whole genome sequencing and HLA genotyping

Genomic DNA was extracted from blood samples using the DNA Blood400 kit (Chemagic) and eluted in 50 µl Elution Buffer (EB; Qiagen). DNA was sheared (Covaris LE220) and sequencing libraries were prepared using the TruSeq Nano DNA HT kit (Illumina Inc.). Libraries were sequenced at Human Longevity (San Diego, CA, USA). 150 bp paired-end whole-genome sequencing (WGS) data were generated to an average read depth of 30× using the HiSeq platform (Illumina X10) and processed using the Burrows Wheeler Aligner (BWA)/Genome Analysis

Toolkit (GATK) best practices pipeline.[29] Short reads were mapped to hg38/GRCh38 (GCA_000001405.15), including alternate assemblies, using an alt-aware version of BWA to generate BAM files.[30] All sequencing data were checked for concordance with data from a SNP Trace Panel (96 markers; Fluidigm) generated before sequencing.

We used the software HLA-HD to infer HLA alleles from WGS data, starting from BAM files generated as described earlier.[31] The MiDAS package for R was used to infer variable amino acids from the HLA allele calls.[32]

Statistical analyses

The association analysis focused on ADA or NAb presence versus absence phenotypes. Logistic regression was used on a per-trial basis to test for association between ADA or NAb, respectively, and HLA alleles with carrier frequencies of at least 0.5% (dominant inheritance model). Patients with ADA that were NAb-negative were excluded from the NAb analyses. No patients with NAb were found for the Impower133 study, which was therefore excluded from the NAb analyses. We included age, sex, five principal components to correct for population stratification (Appendix S1: Figure S1), and trial arm (where applicable) as covariates. We used a multi-degree-of-freedom omnibus test to test for association at multiallelic amino acid positions. For the meta-analyses, the random-effects model method in the R package "meta" was used to calculate effect estimates, 95% confidence intervals, p values, and between-study variance using the DerSimonian–Laird method.[33] No significant heterogeneity between studies was identified. Edginton's method (sum of p), as implemented in the R package "metap", was used for meta-analysis of omnibus test results for variable amino acid positions. The Bonferroni method was applied to correct p values for multiple testing, correcting for the number of included HLA alleles or variable amino acid positions, respectively. In the case of statistically significant results, we performed step-wise conditional analyses, adding the top-associated variable as a covariate to the regression model. The p values are reported uncorrected, and the respective numbers of tests and significance thresholds (4.5×10^{-4}–1.42×10^{-4}) are reported in Appendix S1: Table S1.

RESULTS

In total, our study included 1982 patients treated with atezolizumab as mono- or combination therapy and with available informed consent for genetic analyses. Of these patients, 591 (29.8%) tested positive for ADA, and we observed a large variability in ADA incidences between treatment combinations and study arms (13.5%–50.3%, Table 1). Samples from the ADA-positive patients were further tested for the presence of NAb, and we found that only a subset of these ($N = 278$; 14.5%) were also NAb-positive (0%–25.5%, Table 1).

In a meta-analysis across the eight studies, we identified a statistically significant association of five HLA class II alleles with the presence of ADA (Appendix S2), with *HLA-DRB1*01:01* showing the strongest association ($p = 3.4 \times 10^{-5}$, OR 1.96; Figure 1, Appendix S1: Figure S2a). The top three alleles, *HLA-DRB1*01:01*, *HLA-DQB1*05:01*, and *HLA-DQA1*01:01,* form a common HLA class II haplotype and are not statistically independent. Indeed, *HLA-DQB1*05: 01* and *HLA-DQA1*01:01* were no longer significantly associated in a conditional analysis including *HLA-DRB1*01:01* as a covariate (Appendix S2). We also inferred variable amino acid positions across all tested HLA genes. Position 96 of HLA-DRß1 showed the strongest significance ($p = 4.8 \times 10^{-5}$), and glutamic acid as the residue at this position was associated with increased risk for ADA (OR 1.91; Figure 1, Appendix S1: Figure S2a). Of all the alleles tested, only *HLA-DRB1*01* alleles *HLA-DRB1*01:01* and *HLA-DRB1*01:02* carried this residue at position 96 (Appendix S1: Table S2), and the amino acid residue did not explain the association better than the top-associated allele (Figure 1). Conditional analysis did not yield further amino acid positions that were independently associated.

Next, we focused on patients with NAb ($N = 278$), excluding individuals with ADA that were not neutralizing from the analysis. The top-associated HLA allele with NAb status was *HLA-DQA1*01:01* ($p = 2.8 \times 10^{-7}$, OR 2.31; Figure 1, Appendix S1: Figure S2b), a member of the same common haplotype that includes *HLA-DRB1*01:01*. In fact, *HLA-DRB1*01:01* was the second strongest associated allele ($p = 1.6 \times 10^{-6}$, OR 2.32) in the NAb analysis (Figure 1, Appendix S2). On amino acid level, position 96 of HLA-DRß1 again showed the strongest association ($p = 3.2 \times 10^{-5}$), with glutamic acid mediating increased risk for NAb development (OR 2.33; Figure 1, Appendix S1: Figure S2b). Conditional analysis revealed no statistically independent associations at the HLA allele or amino acid level.

Both ADA and NAb analyses yielded associations of the same group of alleles and amino acid positions, suggesting that the ADA associations are explained by the subset of patients exhibiting NAb. We therefore also investigated possible HLA associations with non-neutralizing ADA only ($N = 313$) and did not obtain significant results on allele or amino acid level after multiple testing correction. *HLA-DRB1*01:01* and *HLA-DQA1*01:01* yielded p values of 0.047 and 0.18, respectively, suggesting that

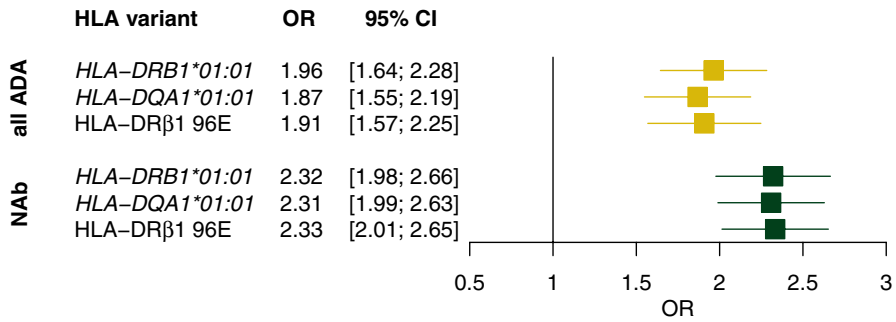

FIGURE 1 Meta-analysis summary results for the top-associated human leukocyte antigen alleles (HLA) and amino acid residue. Forest plot showing the meta-analysis association effect estimates of the top-associated HLA alleles *HLA-DRB1*01:01* and *HLA-DQA1*01:01*, as well as amino acid residue 96E (glutamic acid) of HLA-DRß1, with antidrug antibody and in vitro neutralizing antibody status, respectively. Bars represent 95% confidence intervals. ADA, antidrug antibodies; HLA, human leukocyte antigen; NAb, in vitro neutralizing antibodies; OR, odds ratio; CI, confidence interval

ADA associations are predominantly driven by NAb status (Appendix S2).

DISCUSSION

Although the formation of ADA is associated with many or most therapeutic antibodies, little is known about the risk factors that predispose individual patients or therapeutic antibodies to their elicitation. Moreover, ADA can exhibit variable effects, with some manifesting as antibodies that do not interfere with therapeutic efficacy, while others are neutralizing in vivo and therefore have greater potential to attenuate efficacy. Given that we were able to analyze a large cohort, our study presents clear evidence for an association of common HLA class II variation with anti-atezolizumab ADA risk. *HLA-DRB1*01:01* and *HLA-DQA1*01:01* were the top-associated alleles for ADA and NAb status, respectively. Both occur together on a common HLA class II haplotype, and each showed statistically significant association with both phenotypes. This is consistent with a single amino acid position and residue (glutamic acid at HLA-DRß1 position 96) being most strongly associated with both ADA and NAb status. Amino acid level associations can be stronger than associations for single alleles if residues are shared between more than one relevant allele, but we did not find evidence for this in our study. Also, position 96 is not located in the peptide binding groove of HLA-DR, and therefore is possibly just a proxy for the allelic association, rather than a causal variant. No significant associations were obtained when considering only patients who exhibited non-neutralizing antibodies. Thus, most of the association with ADA can be explained by the NAb subset. Some 47.2% of ADA-positive patients (278/591) carried detectable antibodies that are neutralizing in vitro, suggesting a certain degree of variation in antibody clonality. The possibility that HLA class II proteins including the associated alpha or beta subunits result in the presentation of a peptide that specifically contributes to NAb formation is consistent with different atezolizumab peptides being presented by different allelic variants of HLA proteins.

Across the eight studies, 81% of patients identified as White/European (Appendix S1: Table S3). Both identified risk alleles show the highest carrier frequencies in White/European reference populations. *HLA-DRB1*01:01* is significantly less common in African American and Chinese individuals, and our study was not powered to assess the role of this allele in these populations separately.[34] Due to the low number of non-European patients, our study was also underpowered to establish possible additional associations of ADA status with HLA alleles that are frequent in such populations, but rare in Europeans. Future studies in more diverse populations could answer this question. To exclude the possibility that our significant results were due to population stratification, we included five genetic principal components as covariates in all statistical analyses.

Effect directionality was consistent across the investigated trials, providing evidence that the associations are specific to the treatment, but independent of cancer indication. In terms of effect estimates, the observed odds ratios in the range of 2 are comparable to reported HLA associations with ADA development for other biologics.[11–13] Carrying a risk allele is neither necessary nor sufficient to predict development of ADA. It is more likely one of many factors that determine ADA risk, and many of these factors remain to be uncovered.[35,36] However, the identification of HLA risk alleles combined with in silico peptide binding prediction might support efforts to identify immunogenic peptides in atezolizumab or possibly other biologics. Different immunogenic peptides are expected to be preferentially presented by different HLA proteins, and so it makes sense that HLA associations are not identical

across published studies, but are specific to the therapeutic antibodies under investigation.

Our study has several limitations, including the already discussed limited heterogeneity of the study population. While the combined cohort size was large enough to detect HLA associations, we did not consider it sufficiently powered for hypothesis-free, genome-wide investigations of common or rare variation, and can therefore not exclude additional genetic risk factors for ADA development. An independent replication of our findings is warranted; however, ADA assays are not routinely done in standard-of-care settings, and it might be challenging to find cohorts of sufficient size.

In conclusion, our results provide evidence that HLA allelic variation can contribute to the development of ADAs in patients treated with therapeutic antibodies. It will be important to assess whether similar associations exist in patient groups treated with other therapeutic antibodies that are known to elicit ADA.

AUTHOR CONTRIBUTIONS

C.H., J.R., L.K., and V.Q. wrote the manuscript; C.H., I.M., and V.Q. designed the research; C.H., J.R., and L.K. performed the research; C.H. analyzed the data; and J.H. contributed new analytical tools.

CONFLICTS OF INTEREST

C.H., J.H., L.K., I.M., and V.Q. are employees of Genentech, Inc./Roche and Roche stockholders. J.R. is a former employee of Roche and a Roche stockholder.

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SUPPORTING INFORMATION

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