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Autoimmune genetic risk variants as germline biomarkers of response to melanoma immune checkpoint inhibition

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Author contributions Vylyny Chat, Robert Ferguson and Tomas Kirchhoff designed the study and drafted the manuscript. Vylyny Chat, Robert Ferguson, Esther Kazlow and Rebecca Lax performed the experiments. Vylyny Chat, Robert Ferguson, Danny Simpson and Tomas Kirchhoff analyzed the data. Una Moran, Anna Pavlick, Dennie Frederick and Genevieve Boland assisted in sample collections and data curations. Ryan Sullivan, Antoni Ribas, Keith Flaherty, Iman Osman, Jeffrey Weber provided the patient specimens, clinical data and clinical resources. Vylyny Chat, Robert Ferguson, Ryan Sullivan, Antoni Ribas, Keith Flaherty, Iman Osman, Jeffrey Weber and Tomas Kirchhoff edited and revised the manuscript. Tomas Kirchhoff led the project. All authors have read and approved the final version of the manuscript.

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Conflict of interest

No conflict of interest, except for:

Antoni Ribas has received honoraria from consulting with Bristol Myers-Squibb, Amgen, Chugai, Genentech, Merck, Novartis and Roche, is in the scientific advisory board of Advaxis, Arcus, Bioncotech, Compugen, CytomX, Five Prime, FLX-Bio, ImaginAb, Isoplexis, Merus and Rgenix, during the conduct of this work was in the scientific advisory board and held stock in Kite-Pharma, and is co-founder of PACT Pharma and Tango Therapeutics.

Ryan Sullivan serves as a Consultant/Advisory Board member at Merck, Amgen, Compugen, Array Biopharma, Novartis, Roche-Genentech and Replimmune, Syndax, and received research support from Merck, Amgen.

Keith Flaherty serves on the Board of Directors of Loxo Oncology, Clovis Oncology, Strata Oncology and Vivid Biosciences; on the Corporate Advisory Boards of X4 Pharmaceuticals and PIC Therapeutics; on the scientific advisory boards of Sanofi, Amgen, Asana, Adaptimmune, Fount, Aeglea, Array BioPharma, Shattuck Labs, Arch Oncology, Tolero, Apricity, Oncoceutics, Fog Pharma, Neon Therapeutics, and Tvardi; and as a consultant to Novartis, Genentech, BMS, Merck, Takeda, Verastem, Checkmate, Boston Biomedical, Pierre Fabre, Cell Medica, and Debiopharm.

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Abstract

Immune-checkpoint inhibition (ICI) treatments improve outcomes for metastatic melanoma; however, >60% of treated patients do not respond to ICI. Current biomarkers do not reliably explain ICI resistance. Given the link between ICI and autoimmunity, we investigated if genetic susceptibility to autoimmunity modulates ICI efficacy. In 436 patients with metastatic melanoma receiving single line ICI or combination treatment, we tested 25 SNPs, associated with > 2 autoimmune diseases in recent genome wide association studies (GWAS), for modulation of ICI efficacy. We found that rs17388568—a risk variant for allergy, colitis and type 1 diabetes—was associated with increased anti-PD-1 response, with significance surpassing multiple testing adjustments (OR =0.26; 95%CI=0.12-0.53; p=0.0002). This variant maps to a locus of established immune-related genes: IL2 and IL21. Our study provides first evidence that autoimmune genetic susceptibility may modulate ICI efficacy, suggesting that systematic testing of autoimmune risk loci could reveal personalized biomarkers of ICI response.

Keywords

Autoimmunity; Germline variants; Immune checkpoint inhibition; Melanoma

INTRODUCTION

Recent introduction of immune-checkpoint inhibition (ICI) treatments has significantly improved survival of patients with metastatic cutaneous melanoma, which historically has accounted for 80% of skin cancer-related mortality [1,2]. ICI induces immune activity by inhibiting two major immune-checkpoint proteins: CTLA-4 (Cytotoxic T-lymphocyte-associated antigen 4) and PD-1 (Programmed cell death protein 1)[3]. While objective response rates vary depending on ICI treatment, a substantial fraction of patients do not respond to ICI (~85 % for anti-CTLA-4 [4–6], ~60% for anti-PD-1 [7,8] and ~50% for combination regimens [9,10]). Moreover, patients can develop severe or even fatal immune-related adverse events (irAEs), with unpredictable patterns [11], often resulting in discontinuation of treatment. There is a pressing need for an identification of reliable biomarkers for a more personalized stratification of patients benefiting from these therapies, which may point to novel molecular pathways to be targeted in improved combination regimens.

The currently proposed biomarkers of response and toxicity, such as serum markers [12,13], tumor mutation burden [14], tumor-associated PDL1 [15], pre-existing CD8+ T cell infiltration in the tumor [16], repertoire of white blood cells (WBC) [17] or host microbiota [18] provide a modest level of predictive power, yet they are hampered by individual

heterogeneity and a lack of specificity. Several studies have recently tested germline variants as more efficient and personalized predictive markers for both response and toxicity in ICI [19,20], in particular with anti-CTLA-4 treatments [21,22]. Our study builds on the notion that ICI antagonizes immune-regulatory pathways and augments immune response for potent tumor surveillance [3]. Since the role of the immune checkpoints in suppressing autoimmunity was being targeted for ICI therapy, it is highly plausible that ICI efficacy is modulated by patients' underlying genetic susceptibility to autoimmunity, as also evidenced by autoimmune-like irAEs commonly observed in ICI [11], the occurrence of which may be associated with increased clinical response [23,11]. The observed link between ICI, irAEs and treatment efficacy leads to our hypothesis that genetic risk factors of autoimmunity may affect ICI response, suggesting their potential utility as biomarkers of more personalized stratification of patients for ICI benefit. In this report, we have utilized findings from recently completed genome-wide association studies (GWAS) on a spectrum of autoimmune diseases [24–26] (See supplementary TableS1 for full references of autoimmune GWAS), and have tested well-established single nucleotide polymorphisms (SNPs) associated with autoimmunity risks in GWAS for their effect on ICI response in a cohort of patients treated by single line or combined ICI.

METHODS

Study population

The patient samples were collected through collaborative efforts among several institutions: New York University Langone Health (NYULH), University of California Los Angeles (UCLA) and Massachusetts General Hospital (MGH). All the patients in the study were treated for metastatic melanoma with immune checkpoint inhibitor therapy; anti-CTLA-4 (ipilimumab, tremelimumab; N=215), anti-PD-1 (nivolumab, pembrolizumab; N=176) and combined anti-CTLA-4/anti-PD-1 (ipilimumab/nivolumab; N=45). Each patient provided blood samples for DNA extraction; demographic and clinical information including sex and age at treatment were also collected. All participants were of self-reported European ancestry.

Response status was assessed in all patients 13 weeks after initiation of treatment using established RECIST 1.1 (Response evaluation criteria in solid tumors) as described previously[27]. In our study, clinical outcomes were classified into two categories: responders and non-responders. Responders consisted of patients with complete response (CR), partial response (PR) and stable disease (SD), while patients with progression of disease (POD) were classified as non-responders.

Selection of Autoimmune Candidate Genetic Variants

From a comprehensive search of the published Genome Wide Association Studies (GWASs) performed on autoimmune diseases, we focused on variants that were associated with at least 3 autoimmune diseases, in which at least one association surpassed the GWAS level of significance ($p=1E-07$). In our selection, we have specifically focused on autoimmune traits that usually manifest as part of irAEs, including allergy, alopecia, ankyloses spondylitis, asthma, celiac disease, colitis, inflammatory bowel disease, multiple sclerosis, pancreatitis,

psoriasis, rheumatoid arthritis, system sclerosis, type 1 diabetes and vitiligo. Our final selection contained 25 SNPs to be tested in this study (Supplementary TableS1).

Genotyping and Quality Control (QC) analysis.

Genomic DNA was isolated from whole blood samples using QiaAmp (Qiagen). The genotyping of 25 selected SNPs was performed using the Sequenom MassArray System (Agena Bioscience Inc,CA) as described elsewhere [28]. Briefly, for quality control (QC), 8 sample duplicates were run along with 2 non-template controls in a 384-well plate. Concordance of duplicates observed was 99% and no cross-contamination was detected. We removed SNPs with genotype information missing in >15% of the samples or SNPs departing Hardy-Weinberg equilibrium ($p < 0.001$). After QC filters, 22, 24 and 23 SNPs remained in the analysis for response to anti-CTLA-4, anti-PD-1 and combination therapy, respectively. Patients with more than 10% of genotype information missing were also removed from the analysis, filtering out 1 anti-CTLA-4 and 1 anti-PD-1 treated patient. Following QC, 214 anti-CTLA-4, 175 anti-PD-1 and 45 combination therapy-treated patients remained in the final analysis.

Statistical Analysis

The statistical differences between patients' demographical characteristics (sex and age) across treatment cohorts were assessed using Chi-square test for categorical variable (sex) and Kruskal-Wallis test for continuous variable (age). Multiple logistic regression was conducted to investigate the association between selected SNPs and ICI response; a major allele was treated as a reference. Responders were defined as controls, while non-responders were classified as cases in all association tests. We used an additive model as a baseline analysis for all logistic regression tests. Where indicated, we also used dominant and recessive genetic models. In the dominant model, the presence of one or two copies of a minor allele was assumed to have the same effect, while in the recessive model, the carriers of at least one major allele were treated as a reference group, against minor allele homozygotes. We performed the analyses in anti-CTLA-4, anti-PD-1 and combination therapy-treated cohorts separately. We also conducted a pooled analysis of patients regardless of treatment types. The effect size of observed associations was reported as odds ratio with 95% confidence interval ($OR \pm 95\%CI$). We employed the Bonferroni method to adjust for multiple testing with a statistical significant threshold of $p < 0.002$. As a secondary analysis, we also tested the interactions of the most significant variants with age and sex using logistic regression. All statistical models were adjusted for age at treatment, sex and treatment drugs (e.g. ipilimumab vs tremelimumab for the anti-CTLA-4 group and nivolumab vs pembrolizumab for the anti-PD-1 group), or treatment types for pooled analysis. Descriptive statistics were performed in R 3.5.1 and all analyses were conducted in PLINK 1.9.

RESULTS

Characteristics of study population

Our study employed 436 metastatic melanoma patients (Table 1). Among these, 215 patients received anti-CTLA-4 (171 ipilimumab and 44 tremelimumab); 176 anti-PD-1 (17

nivolumab, 159 pembrolizumab) and 45 combination therapies (anti-CTLA-4 and anti-PD-1). For anti-CTLA-4, 150 samples were obtained from patients treated at NYULH; 50 at UCLA and 15 at MGH. For anti-PD-1, 77 samples were obtained from patients treated at NYULH, 84 at UCLA and 15 at MGH; while for combination therapy (anti-CTLA-4/antiPD-1), 34 samples were obtained from patients at NYULH and 11 at MGH (Table1). The overall median age at treatment was 62.85 years (range: 19-90.20). A larger proportion of our patients were males (67%), consistent with the sex distribution of metastatic melanoma. The response rate for combination therapy was the highest (76.74%). For anti-CTLA-4- treated patients, the overall response rate was 36.91 %, while anti-PD-1 had 52.04% response rate. Some statistically significant difference has been observed between treatment cohorts for age of treated patients ($p=0.02$) (Table 1). Therefore, in subsequent analyses the effect of SNPs on ICI efficacy was tested separately in anti-CTLA, anti-PD1 and combination-therapy treated cohorts.

Analysis of association of 25 autoimmune risk SNPs with response in anti-CTLA-4

A total of 22 SNPs that passed QC (quality control) were tested for their association with response in 213 anti-CTLA-4 treated patients. Among those patients, 78 patients were responders (controls) and 135 were non-responders (cases) (Table 2). Both additive and recessive logistic regression models revealed several associations with response; however, none of these reached a level of statistical significance corrected for multiple testing using the Bonferroni adjustment ($p<0.002$) (supplementary Table S2), or a less stringent Holm-Bonferroni method. The most significant association with response in anti-CTLA-4-treated patients was found for rs1893217 under a dominant logistic model: our data showed that the carriers of at least one copy of a minor allele G (AG or GG) were 2.79 times more likely to be non-responders, compared to those with the homozygous reference genotype (AA) (95%CI= 1.36-5.73; $p=0.005$; Table 2). rs1893217 was mapped to the *PTPN2* gene, and is associated with autoimmune diseases such as celiac disease, inflammatory bowel disease, rheumatoid arthritis and type 1 diabetes (Supplementary Table S1). While this was the most significant association in the anti-CTLA-4 analysis, the statistical significance was marginal after adjustment for multiple testing by Bonferroni or Holm-Bonferroni methods (p -adjusted = 0.09). We also tested whether the association of rs1893217 with anti-CTLA-4 response was modified by age and sex and while the effect of rs1893217 was stronger in the younger age group (OR=6.43, 95% CI 1.7423.82), none of the interactions with age or sex was statistically significant (Figure S1).

Analysis of association of 25 autoimmune risk SNPs with response in anti-PD-1

For anti-PD-1 treatment, 24 SNPs passing QC were tested in 169 patients, of whom 88 were responders (controls) and 81 were non-responders (cases) (Table 2). An additive model revealed the most significant association with response for rs17388568 (OR=0.38; 95%CI=0.21-0.67; $p=0.0008$; Table 3; Full results in supplementary Table S2), significantly surpassing the Bonferroni adjustment for multiple testing ($p<0.002$). Under the dominant regression model, consistent with the additive model, rs17388568 was found to have the strongest association with response (OR=0.26; 95%CI=0.12-0.53; $p=0.0002$; Table2), surpassing the Bonferroni multiple testing adjustment. In this analysis, carriers of at least one minor allele (GA or AA) of rs17388568 are 74% less likely to resist anti-PD-1

treatment, compared to those with GG genotype. rs17388568 was mapped to the genomic region containing IL2, ADAD1 and IL21, a locus previously associated with allergy, colitis and type 1 diabetes (Supplementary Table S1). The interaction analyses with age and sex for rs17388568 showed stronger effects in the younger female patients; yet, none of these associations was statistically significant (Figure S2).

Analysis of association of 25 autoimmune risk SNPs with response in combination therapy

In a cohort of 43 combination-therapy treated patients (N responders=controls=33, N non-responders=cases=10; Table2), none of the associations with the 23 SNPs tested reached statistical significance after adjustments for multiple testing with both the Bonferroni and Holm-Bonferroni methods (Full results Supplementary Table S2). However, due to the small sample size in the combination-therapy cohort, this analysis may have a limited power as evidenced by wide confidence intervals (Table2). Hence, the findings should be interpreted with caution. Finally, the pooled association analysis has been performed across treatments, grouping anti-CTLA-4, anti-PD-1 and combination-therapy treated patients together adjusted for age, sex and treatment drugs; no significant association with treatment response was observed (Supplementary Table S2).

DISCUSSION

Our study provides the first targeted analysis of the association of GWAS autoimmune germline genetic susceptibility loci and treatment efficacy in melanoma patients receiving immune-checkpoint inhibition (ICI). While there have been a handful of previous small-scale reports assessing the role of immune-related genetic variants in immunotherapy response [19–22], our study included relatively large cohorts of metastatic melanoma patients across different treatments and for the first time explored a link between genetic susceptibility to autoimmunity with ICI treatment outcomes. While only 25 SNPs were included in the analysis of our study, this subset was derived in a specifically focused manner, only targeting GWAS-level variants that were reproducibly associated with 3 or more autoimmune conditions, in particular those conditions that were similar to manifestations of ICI-related immune toxicity. Our analyses revealed significant associations with treatment efficacy, particularly for patients treated by anti-PD-1 (nivolumab, pembrolizumab).

The most significant association in our study was observed for rs17388568 with response to anti-PD-1. Our data showed that the carriers of a minor allele (A) of rs17388568 were 74% less likely to resist anti-PD-1 treatment (Table2). By inverting the odds ratio, the findings suggested that the minor allele A was associated with an increased ICI response; the carriers of A allele are 3.84 times more likely to respond to anti-PD-1 therapy ($OR=1/0.26=3.84$). This association was observed in both additive and dominant models with comparable association effects ($OR=0.38$; $p=0.0008$ and $OR=0.26$; $p=0.0002$, respectively) (Table2, Table3). In several autoimmune GWAS, a minor allele of rs17388568 (A) was found to be associated with higher risk of autoimmunity such as allergy, colitis and type 1 diabetes [24–26] (Table4), suggesting a potential association of the A allele with increased immune activity. This is consistent with our findings showing that the carriers of the A allele of

rs17388568 (susceptible for the risk of autoimmunity) associate with improved anti-PD-1 response. rs17388568 maps in the locus containing three candidate genes: ADAD1, IL2 and IL21 (Supplementary TableS1). Despite the direct mapping of rs17388568 in the intron of ADAD1 (adenosine deaminase containing domain 1), this candidate gene is mainly involved in testis development, with unclear functional relevance to autoimmunity [29] or immune response in general. In contrast, IL2 and IL21 are both important signaling cytokines in adaptive immunity and previous analyses showed that both genes are contained within a linkage disequilibrium block with rs17388568, suggesting a possible correlation with other variants or functional effects in these two interleukin genes [29]. IL2 is required for activation of both Th1 and Treg cells [30]. While a direct link between IL-2 and response to anti-PD-1 therapy has not been systematically investigated, emerging evidence suggests that IL-2 production could overcome the PD-1-PDL-1 inhibitory pathway [31] and can stimulate cytotoxic CD8+ T-cells, one of the positive markers of anti-PD-1 response [32]. Given this evidence, it is likely that the putative mechanism of the associations between rs17388568 and anti-PD-1 efficacy observed in this study is through IL2-mediated regulation of immune homeostasis, possibly upregulating the production of CD8+ T cells. Interestingly, IL-2 monotherapy was originally approved for melanoma due to its association with induced tumor regression [32], potentially through the increased CD8+ T cell activation. Additionally, there is an ongoing clinical trial for a combined IL2 and anti-PD-1 treatment for metastatic renal cell carcinoma (Trial identifier: NCT02989714).

Another gene in the vicinity of rs17388568 is IL21 that also belongs to the gamma cytokine family [30]. Numerous studies have reported the role of IL-21 in innate and adaptive immunity through the activation of JAK/STAT pathways [33,34] and interestingly, patients with autoimmune diseases show elevated IL21 levels [34,35]. IL-21 was also found to induce PD-1 expression in T cells [36], what may be a potential explanation of our observed association of rs17388568 with anti-PD-1 sensitivity. Several studies have observed suggestive clinical efficacy of combined IL-21 and anti-PD-1 therapy in preclinical mouse models, which was correlated with elevated tumor infiltrated CD8+ T cells [37] and there are ongoing phase I clinical trials of anti-PD-1 and IL-21 for solid tumors [38]. While these and other evidence clearly point to a potential biological link between rs17388568 and IL-2/IL21 genes in the locus, more detailed fine-mapping analysis of these associations will be needed. In particular, the correlation of rs17388568 and other potential variants in this region with gene expressions of both candidates will be needed to provide further functional evidence of the involvement of the genetic variants in this locus in modulation of anti-PD-1 efficacy.

Other associations were observed in this study, in particular for rs1893217 with response to anti-CTLA-4 ICI (OR=2.79; 95%CI= 1.36-5.73; p=0.005; Table 2). rs1893217 was mapped to PTPN2 (protein tyrosine phosphatase non-receptor type 2). PTPN2 is a negative regulator in the JAK/STAT cascade inhibiting downstream cytokine signaling[39]. Current research in anti-PD-1 treated mice suggested an association between PTPN2 deletions with improved response[40]. While promising, however, the statistical significance in our study is borderline, and additional larger validation scanning will be needed to confirm these observations.

Our data did not find statistically significant interactions of the observed associations with age and sex. However, given recent suggestive evidence that age and sex modify ICI outcomes [41,42], these will need to be explored in larger patient cohorts.

In summary, we found a significant association of autoimmune genetic variants with ICI efficacy, particularly for anti-PD-1 treated patients, with a level of statistical significance surpassing adjustments for multiple testing. The findings generated here will be substantially enhanced by functional studies in order to fully understand the directionality and mechanisms of action of associated autoimmune genetic variants in the context of ICI outcome. Despite the current lack of such knowledge, our findings offer valuable insights on genetic markers potentially stratifying patients by treatment benefits using a simple method of SNP genotyping. These observations may stimulate more systematic efforts to assess genetic variants as putative personalized predictive biomarkers of ICI outcomes, by employing larger patient subsets from clinical trials and standard-of-care protocols, coupled with denser SNP arrays in an immune-related format or a genome-wide context. These efforts may substantially complement recent personalized strategies integrating known biomarkers of ICI outcomes, such as those proposed as part of cancer immunograms [43]. A limitation of this report is that we did not assess immune-related toxicity in our patients, which is very relevant given the link to autoimmune genetic susceptibility. As the focus of the current study was on markers of ICI efficacy, the assessment of germline genetic risk variants of autoimmunity in relation to irAEs will be tested in a subsequent study in this patient cohort as well as expanded patient populations from multi-institutional collaborations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CI	Confidence interval
GWAS	Genome-wide association study
ICI	Immune-checkpoint inhibition
irAEs	Immune-related adverse events
MGH	Massachusetts General Hospital
NYULH	New York University Langone Health
OR	Odds ratio
PTPN2	Protein tyrosine phosphatase non-receptor type 2

QC	Quality control
SNP	Single nucleotide polymorphism
UCLA	University of California Los Angeles

REFERENCES

1. Society AC (2015) Cancer Facts & Figure 2015. Atlanta: American Cancer Society
2. Garbe C, Eigentler TK, Keilholz U, Hauschild A, Kirkwood JM (2011) Systematic review of medical treatment in melanoma: current status and future prospects. *The oncologist* 16 (1):5–24 [PubMed: 21212434]
3. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 12 (4):252–264 [PubMed: 22437870]
4. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen T-T, Berman DM, Wolchok JD (2015) Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol* 33 (17):1889–1894 [PubMed: 25667295]
5. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G (2013) Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol* 31 (5):616–622 [PubMed: 23295794]
6. Hodi FS, O'day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* (363):711–723 [PubMed: 20525992]
7. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, Hodi FS, Schachter J, Pavlick AC, Lewis KD, Cranmer LD, Blank CU, O'Day SJ, Ascierto PA, Salama AK, Margolin KA, Loquai C, Eigentler TK, Gangadhar TC, Carlino MS, Agarwala SS, Moschos SJ, Sosman JA, Goldinger SM, Shapira-Frommer R, Gonzalez R, Kirkwood JM, Wolchok JD, Eggermont A, Li XN, Zhou W, Zernhelt AM, Lis J, Ebbinghaus S, Kang SP, Daud A (2015) Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *Lancet Oncol* 16 (8):908–918. doi:10.1016/S1470-2045(15)00083-2 [PubMed: 26115796]
8. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, Hoeller C, Khushalani NI, Miller WH Jr., Lao CD, Linette GP, Thomas L, Lorigan P, Grossmann KF, Hassel JC, Maio M, Sznol M, Ascierto PA, Mohr, Chmielowski B, Bryce A, Svane IM, Grob JJ, Krackhardt AM, Horak C, Lambert A, Yang AS, Larkin J (2015) Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 16 (4):375–384. doi:10.1016/S1470-2045(15)70076-8 [PubMed: 25795410]
9. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon R, A, Reed K (2013) Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 369 (2):122–133 [PubMed: 23724867]
10. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P (2015) Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* 373 (1):23–34 [PubMed: 26027431]
11. Bertrand A, Kostine M, Truchetet M- E, Schaefferbeke T, Barnette T (2015) Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. *BMC medicine* 13 (1):211 [PubMed: 26337719]
12. Schindler K, Harmankaya K, Kuk D, Mangana J, Michielin O, Hoeller C, Dummer R, Pehamberger H, Wolchok JD, Postow MA (2014) Correlation of absolute and relative eosinophil counts with immune-related adverse events in melanoma patients treated with ipilimumab. *American Society of Clinical Oncology*,
13. Tarhini AA, Sander C, Zahoor H, Kirkwood JM, Butterfield LH, Malhotra U, Lin Y (2015) Baseline circulating IL-17 predicts toxicity while TGF- β 1 and IL-10 are prognostic of relapse in

ipilimumab neoadjuvant therapy of melanoma. *J Immunother Cancer* 3 (1):39 [PubMed: 26380086]

14. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS (2014) Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 371 (23):2189–2199 [PubMed: 25409260]
15. Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Calì A, Cuppone F, Sperduti I, Giannarelli D, Chirosi M (2015) Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PloS one* 10 (6):e0130142 [PubMed: 26086854]
16. Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nat* 515 (7528):568
17. Fujisawa Y, Yoshino K, Otsuka A, Funakoshi T, Fujimura T, Yamamoto Y, Hata H, Goshio M, Tanaka R, Yamaguchi K (2017) Fluctuations in routine blood count might signal severe immune-related adverse events in melanoma patients treated with nivolumab. *J Dermatol Sci* 88 (2):225–231 [PubMed: 28736218]
18. Gopalakrishnan V, Spencer C, Nezi L, Reuben A, Andrews M, Karpinets T, Prieto P, Vicente D, Hoffman K, Wei S (2018) Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359 (6371):97–103 [PubMed: 29097493]
19. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, Guida M, Hyams DM, Gómez H, Bastholt L (2011) A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. *J Transl Med* 9 (1):204 [PubMed: 22123319]
20. Berman D, Parker SM, Siegel J, Chasalow SD, Weber J, Galbraith S, Targan SR, Wang HL (2010) Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. *Cancer Immunity Archive* 10 (1): 11
21. Breunis WB, Tarazona-Santos E, Chen R, Kiley M, Rosenberg SA, Chanock SJ (2008) Influence of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) common polymorphisms on outcome in treatment of melanoma patients with CTLA-4 blockade. *J Immunother* (Hagerstown, Md: 1997) 31 (6):586
22. Queirolo P, Morabito A, Laurent S, Lastraioli S, Piccioli P, Ascierto P, Gentile G, Serra M, Marasco A, Tornari E (2013) Association of CTLA-4 polymorphisms with improved overall survival in melanoma patients treated with CTLA-4 blockade: a pilot study. *Cancer Invest* 31 (5): 336–345 [PubMed: 23641913]
23. Attia P, Gao PQ, Michael YJ, Rosenberg SA (2005) Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-CTLA-4. *AACR*,
24. Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, St Pourcain B, Ring SM, Mountain JL, Franke U (2013) A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nat Genet* 45 (8):907 [PubMed: 23817569]
25. Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, Lee JC, Goyette P, Imielinski M, Latiano A (2011) Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 43 (3):246–252 [PubMed: 21297633]
26. Consortium WTCC (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nat* 447 (7145):661
27. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45 (2):228–247 [PubMed: 19097774]
28. Rendleman J, Vogelsang M, Bapodra A, Adaniel C, Silva I, Moogk D, Martinez CN, Fleming N, Shields J, Shapiro R (2015) Genetic associations of the interleukin locus at 1q32. 1 with clinical outcomes of cutaneous melanoma. *J Med Genet:jmedgenet-2014-102832*

29. Chistiakov DA, Voronova NV, Chistiakov PA (2008) The crucial role of IL-2/IL-2RA-mediated immune regulation in the pathogenesis of type 1 diabetes, an evidence coming from genetic and animal model studies. *Immunol Lett* 118 (1):1–5 [PubMed: 18417224]
30. Sim GC, Radvanyi L (2014) The IL-2 cytokine family in cancer immunotherapy. *Cytokine & Growth Factor Rev* 25 (4):377–390 [PubMed: 25200249]
31. Carter LL, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR, Collins M, Honjo T, Freeman GJ, Carreno BM (2002) PD- 1: PD- L inhibitory pathway affects both CD4+ and CD8+ T cells and is overcome by IL- 2. *Eur J Immunol* 32 (3):634–643 [PubMed: 11857337]
32. Hughes T, Klairmont M, Sharfman WH, Kaufman HL (2015) Interleukin-2, ipilimumab, and anti-PD-1: clinical management and the evolving role of immunotherapy for the treatment of patients with metastatic melanoma. *Cancer Biol Ther* (just-accepted):00-00
33. Di Carlo E, De Toter D, Piazza T, Fabbi M, Ferrini S (2007) Role of IL-21 in immune-regulation and tumor immunotherapy. *Cancer Immunol Immunother* 56 (9):1323–1334 [PubMed: 17447063]
34. Monteleone G, Monteleone I, Fina D, Vavassori P, Blanco GDV, Caruso R, Tersigni R, Alessandroni L, Biancone L, Naccari GC (2005) Interleukin-21 enhances T-helper cell type I signaling and interferon- γ production in Crohn's disease. *Gastroenterol* 128 (3):687–694
35. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK (2007) IL-21 initiates an alternative pathway to induce proinflammatory T H 17 cells. *Nat* 448 (7152):484
36. Kinter AL, Godbout EJ, McNally JP, Sereti I, Roby GA, O'Shea MA, Fauci AS (2008) The common γ -chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. *J Immunol* 181 (10):6738–6746 [PubMed: 18981091]
37. Lewis KE, Selby MJ, Masters G, Valle J, Dito G, Curtis WR, Garcia R, Mink KA, Waggle KS, Holdren MS (2018) Interleukin-21 combined with PD-1 or CTLA-4 blockade enhances antitumor immunity in mouse tumor models. *Oncoimmunology* 7 (1):e1377873
38. Chow LQM, Gordon MS, Logan TF, Antonia SJ, Bhatia S, Thompson JA, Brahmer JR, Solberg G, Bittner R, Fontana D (2013) Phase I dose escalation study of recombinant interleukin-21 (rIL-21; BMS-982470) in combination with nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with advanced or metastatic solid tumors. American Society of Clinical Oncology,
39. Simoncic PD, Lee-Loy A, Barber DL, Tremblay ML, McGlade CJ (2002) The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3. *Curr Biol* 12 (6):446–453 [PubMed: 11909529]
40. Manguso RT, Pope HW, Zimmer MD, Brown FD, Yates KB, Miller BC, Collins NB, Bi K, LaFleur MW, Juneja VR (2017) In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nat* 547 (7664):413
41. Kugel CH, Douglass SM, Webster MR, Kaur A, Liu Q, Yin X, Weiss SA, Darvishian F, Al-Rohil RN, Ndoye A (2018) Age correlates with response to anti-PD1, reflecting age-related differences in intratumoral effector and regulatory T-cell populations. *Clin Cancer Res* 24 (21):5347–5356 [PubMed: 29898988]
42. Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD, Goldhirsch A (2018) Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol* 19 (6):737–746 [PubMed: 29778737]
43. Blank CU, Haanen JB, Ribas A, Schumacher TN (2016) The “cancer immunogram”. *Science* 352(6286):658–660 [PubMed: 27151852]

Table 1:

Patients' characteristics by treatment types

Characteristics	Treatment Types				p-value ^c
	Total	Anti-CTLA-4 (Ipilimumab or tremelimumab)	Anti-PD-1 (Nivolumab or pembrolizumab)	Combination (anti-CTLA-4+anti-PD-1)	
Median age (Range)	62.85(19-90.20)	59.95(23-90.20)	65.80(19-90)	61.90(20-89)	0.02
Sex					
Male (%)	292(66.97)	145(67.44)	119(67.61)	28(62.22)	0.77
Response outcome ^b					
Response (%)	201(46.96)	79(36.91)	89(52.04)	33(76.74)	
Institutions (N, %)					
NYULH ^a	261(59.86)	150(69.76)	77(43.75)	34(75.55)	
UCLA	134(30.73)	50(23.25)	84(47.72)	0(0.00)	
MGH	41(9.41)	15(6.99)	15(8.53)	11(24.44)	
Total	436	215	176	45	

^aOne patient was later removed in the quality control (QC) step for anti-CTLA-4 and anti-PD-1

^bThere is 1 missing value in anti-CTLA-4, 6 in anti-PD-1 and 2 in combination therapy

^cRelevant patients' characteristics (age, sex) were tested for statistical significance between treatment types. Kruskal-Wallis test was performed for the continuous variable (age); while Chi-square test was used to test the statistical difference for the categorical variable (sex).

The top 3 most significant associations with response to ICI under dominant logistic regression models^a

Table2:

SNPs	Reported Genes	Chromosome position	Major/Minor allele	Anti-CTLA-4 (N Controls= 78, N Cases= 135) (N total= 213)		Anti-PD-1 (N Controls= 88, N Cases= 81) (N total= 169)		Combined therapy (N Controls= 33, N Cases= 10) (N total= 43)	
				OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
rs10488631	TNPO3, IRF5	chr7:128954129	T/C	1.14(0.52-2.49)	0.74	1.20(0.55-2.60)	0.63	31.19(1.62-597.9)	0.02
rs17388568	IL2, ADAD1, IL21	chr4:122408207	G/A	NA	NA	0.26(0.12-0.53)	0.0002 *	0.94(0.14-6.05)	0.95
rs1893217	PTPN2	chr18:12809341	A/G	2.79(1.36-5.73)	0.005	1.56(0.76-3.16)	0.21	6.95(1.06-45.26)	0.04
rs2111485	FAP, IFIH1	chr2:162254026	G/A	0.62(0.32-1.19)	0.15	0.96(0.49-1.87)	0.91	0.21(0.04-0.98)	0.04
rs2187668	HLA-DQA1	chr6:32638107	C/T	1.36(0.66-2.78)	0.39	2.14(1.06-4.31)	0.03	1.15(0.15-8.48)	0.88
rs2476601	PHTF1, PTPN22	chr1:113834946	G/A	3.17(1.02-9.85)	0.04	0.36(0.09-1.48)	0.16	1.52(0.11-21.01)	0.75
rs6679677	PHTF1, PTPN22	chr1:11376n86	C/A	2.95(1.14-7.60)	0.02	0.59(0.21-1.61)	0.30	1.52(0.11-21.01)	0.75

^aModels adjusted for age, sex and treatment drug (ipilimumab or tremelimumab; nivolumab or pembrolizumab)

Controls: ICI responders; Cases: ICI non-responders

NA refers to SNPs removed in QC step; the top three SNPs in each treatment cohort are bolded.

* Asterisk indicates p-value surpassing the Bonferroni multiple testing adjustment (p<0.002).

Table3:

Associations of rs1893217 and rs17388568 with response in anti-CTLA-4 and anti-PD-1 under different genetic models^a

	Additive Model		Dominant Model		Recessive Model		
	OR(95%CI)	p-value	OR(95%CI)	p-value	OR(95%CI)	p-value	
Anti-CTLA-4 ^b rs1893217	Reference	Reference	Reference	Reference	Reference	Reference	
	AA	2.26(1.21-4.20)	0.01				
	AG	5.11(2.97-9.51)	0.01	2.79(1.36-5.73)	0.005	1.89(0.35-10.11)	0.45
	GG						
Anti-PD-1 ^c rs17388568	Reference	Reference	Reference	Reference	Reference	Reference	
	GG	0.38(0.21-0.67)	0.0008 [*]	0.26(0.12-0.53)	0.0002 [*]	0.38(0.11-1.33)	0.13
	GA	0.14(0.08-0.25)	0.0008 [*]				
	AA						

^aModels adjusted for age, sex and treatment drug (ipilimumab or tremelimumab; nivolumab or pembrolizumab)

^bN Controls= 78, N Cases= 135 (Controls: ICI responders; Cases: ICI non-responders); for the dominant model: the genotype group comparisons were as follows: AA (reference) vs AG/GG; for the recessive model: AA/AG(reference) vs GG

^cN Controls= 88, N Cases= 81 (Controls: ICI responders; Cases: ICI non-responders); for the dominant model: the genotype group comparisons were as follows: GG (reference) vs GA/AA; for the recessive model: GG/GA(reference) vs AA

^{*} Asterisk indicates p-value surpassing the Bonferroni multiple testing adjustment (p<0.002).

Table4:
Reported associations of rs17388568 with autoimmune diseases from genome wide association studies (GWAS)

SNPs/Associated Traits	Reported Genes	Major/Minor Allele	OR(95%CI)	p-value	Reference
rs17388568					
Allergy	IL2,		1.08(1.05-1.10)	3.90E-08	[24]
Colitis	ADAD1,	G/A	1.12 (1.07-1.17)	9.49E-07	[25]
Type 1 diabetes	IL21		1.58(1.27-1.95)	3.27E-06	[26]