PNAS LETTER

Reply to Bousdar et al.: Common inherited loss-of-function mutations in the innate sensor *NOD2* contribute to exceptional immune response to cancer immunotherapy

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We thank Bousdar et al. for their feedback and welcome their findings from analyzing inherited *NOD2* variants in their cohort of 117 patients with metastatic non–small cell lung cancer treated with anti–PD-1/PD-L1 at two Spanish centers, assessing toxicity and response at 3, 6, and 12 mo. We note important differences compared to the analysis by Barnet et al. particularly with respect to the definition of exceptional responder patients and the definition of *NOD2* loss-of-function alleles.

Bousdar et al. have defined "exceptional responders" differently from the definition in Barnet et al., PNAS 2025. In Barnet et al. it was the occurrence of at least one grade 2 or greater immune-related adverse event (irAE) AND progression-free survival of at least 2 y on single-agent anti-PD1/PDL1. In Bousdar et al., they have analyzed all patients with or without irAEs, and cancer response/nonresponse was not clearly defined and only followed to 12 mo after the start of treatment. It is also not specified whether the Spanish cohort received single-agent PD1/PD-L1 or combination with chemotherapy. Patients were recruited after seminal trials (KEYNOTE-189 & IMpower-150; refs. 1 and 2) redefined first-line standard of care as chemotherapy combined with PD1/PD-L1, presumably impacting treatment decisions.

Figure 4*A* in Barnet et al. analyses an expanded cohort of 103 individuals where only 63 patients experienced a grade 2 or greater irAE, and analyses survival for a median 48 mo in those experiencing an irAE. Inheriting a *NOD2* loss-of-function allele was associated with improved survival only in those individuals experiencing an irAE. *SI Appendix*, Table S11 of Barnet et al. observes the same trend in a basket study cohort of 100 individuals with a range of cancers treated with anti-PD1/PDL1, where inheriting a NOD2 loss-of-function allele was associated with cancer response in the 24 patients who experienced an irAE but not in the 76 patients who did not experience a grade 2 or greater irAE.

Barnet et al. noted the absence of association between inherited *NOD2* loss-of-function and cancer survival in a third cohort where irAE data were not available: "we were able to access a public dataset of 202 exome sequences from NSCLC patients treated with single-agent anti-PD(L)1 (SU2C-MARK). This cohort did not show a significant difference in survival outcomes according to NOD2 loss-of-function genotype, although information regarding irAEs was not available to identify the subset of patients who experienced irAEs." Barnet et al. also noted the absence of association *NOD2* genotype with outcomes in a cohort treated with chemotherapy in combination with PD1/PD-L1: "analysis of these combination-treated cohorts showed no significant effect of *NOD2* genotype on clinical outcome.... Speculatively, chemotherapy might perturb the intestinal barrier or augment cancer immunogenicity in other ways to obviate the beneficial effect of *NOD2* loss-of-function."

It would be interesting to refine the analysis of the cohort analyzed by Bousdar et al. into those receiving single-agent treatment and those experiencing a grade 2 or greater irAE and those who did not, to determine whether or not the results are similar to Barnet et al., figure 4*A* and *SI Appendix*, Table S11. We acknowledge that this will require survival data for the Spanish cohort for 50 mo or more, substantially beyond the 12-mo follow-up used by Bousdar et al. here.

The third important difference between the two studies is the definition of *NOD2* loss-of-function alleles. In Barnet et al., analysis was restricted to a set of 33 inherited *NOD2* loss-of-function variants that had previously been demonstrated experimentally to impair NOD2 signaling to NF-κB reporter genes. As such, it is not the case that Barnet et al. did not find variants such as p.V908L, p.T596M, and p.V793. Rather, that analysis was intentionally restricted to demonstrate LOF variants.

By contrast, Bousdar et al. have analyzed all inherited *NOD2* variants, without focusing on those that have been biochemically demonstrated to cause loss-of-function. It would be interesting to refine their analysis by limiting it to the 33 experimentally defined loss-of-function variants in table 1 of Barnet et al., which includes four of the variants reported in figure 1.

We look forward to learning whether or not there is a similar association between inherited *NOD2* loss-of-function variants, occurrence of irAEs, and cancer response to PD1-blockade in the independent cohort cared for by Bousdar et al.

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The authors declare no competing interest.

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