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Letter to *Blood*

TO THE EDITOR:

IDH-mutant myeloid neoplasms are associated with seronegative rheumatoid arthritis and innate immune activation

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High prevalence of *IDH* mutations in seronegative rheumatoid arthritis (RA) with myeloid neoplasm, elevated 2-hydroxyglutarate, dysregulated innate immunity, and proinflammatory microenvironment suggests causative association between *IDH* mutations and seronegative RA. Our findings merit investigation of *IDH* inhibitors as therapeutics for seronegative *IDH*-mutated RA.

Autoimmune manifestations including autoimmune rheumatic disease (AIRD) are reported in 8% to 30% of patients with myeloid neoplasm (MN), including myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML), and myeloproliferative neoplasms (MPNs).[1-4] Furthermore, the risk of developing MN is elevated in autoimmune diseases.[5,6] However, the mechanisms underpinning the association between autoimmune disease and MN remain unclear.

We assessed the burden of AIRD in patients with MN (n = 1702) including MDS (n = 861), AML (n = 640), MDS/MPN (n = 112), and MPN (n = 89). AIRD was identified using the International Classification of Diseases, which was then verified by experienced rheumatologists (supplemental Figure 1A-B and supplemental Table 1, available on the *Blood* website). The study was approved by the respective ethics committees and performed in accordance with the Declaration of Helsinki. Methodological details are provided in the supplemental materials.

The median age of the cohort was 68 years (interquartile range, 59-75), 1023 (60%) of the subjects were male (supplemental Table 2), and 7.7% of patients with MN had AIRD (MN-AIRD). Enrichment of AIRD was observed in MDS, MDS/MPN, and MPN compared with AML (9.5%, 9.8%, 10.1%, and 4.7%, respectively; $P = .003$) (Figure 1A). MN cohort displayed male predominance (60.1% vs 39.9%), but a higher proportion of females had concomitant MN-AIRD (55.7% vs 44.3%; $P < .01$). This was confirmed when analyzed by sex (11% female vs 6% male, $P < .0001$), especially in MDS-AIRD (supplemental Figure 1C-E). Inflammatory arthritis (n = 64, 48.9%), inflammatory connective tissue diseases (n = 23, 17.2%), polymyalgia rheumatica (n = 18, 13.7%), and vasculitis (n = 16, 12.2%) were the most prevalent AIRD (Figure 1B). Notably, 85.9% of inflammatory arthritis diseases were rheumatoid arthritis (RA) (n = 55) followed by peripheral (n = 8, 12.5%) and axial (n = 1, 1.6%) spondyloarthropathies.

Enrichment for somatic mutations in the epigenetic modifiers *DNMT3A* (odds ratio 1.93, $P = .02$) and *IDH1/2* (odds ratio 1.9, $P = .02$) was observed in MN-AIRD (Figure 1C). To investigate this observed link, we analyzed an expanded cohort of 1356 patients with MN screened for *IDH1/2* mutations. Enrichment of *IDH1/2* mutations was identified in all MN-AIRD cases compared with non-AIRD cases (18.8% vs 11.3%; $P = .04$) driven primarily by the enrichment noted in MDS-AIRD (20.7% vs 6.9%; $P = .001$), suggesting a link between these somatic mutations and autoimmune features (Figure 1D). A similar trend was observed in AML (22.7% vs 18.7%; $P = .58$) but did not reach statistical significance in part due to the higher prevalence of seropositive RA in AML compared with MDS (78% vs 29%; $P = .03$), and the lack of association of seropositive RA with *IDH* mutation. Unexpectedly, males with MN-AIRD displayed *IDH1/2* mutations enrichment when compared with those without AIRD (23% vs 11%; $P = .01$), which was not apparent in females (14% vs 12%; $P = .67$) (Figure 1E).

We next evaluated AIRD subtypes in patients with *IDH1/2* mutations. *IDH1/2* mutations were increased in MN with RA vs other AIRD (30.6% vs 11.7%; $P = .03$) (Figure 1F-G) with striking enrichment in seronegative vs seropositive RA (44.4% vs 0%; $P = .009$) (Figure 1H). Although *IDH1/2* mutations occurred with similar frequency in AIRD, we found enrichment of *IDH1* mutations in seronegative RA (75% *IDH1* vs 25% *IDH2*), and *IDH2* mutations were prevalent in other AIRD (100% *IDH2* vs 0%

IDH1; *P* = .009) (Figure 1I). The higher frequency of AIRD was verified in an independent validation cohort of patients with CMML (n = 21) who were selected based on *IDH* mutation status, irrespective of AIRD status. Of the patients with *IDH1/2*-mutated CMML, 62% (n = 13) had AIRD (n = 7) or other autoinflammatory manifestations (n = 6) (Figure 1J). Our findings indicate enrichment of *IDH1* and *IDH2* mutations in AIRD, particularly seronegative RA.

If *IDH1/2* mutations play a pathogenic role in AIRD, it is plausible that mutant clones precede AIRD diagnosis. We therefore analyzed the temporal events surrounding mutation and

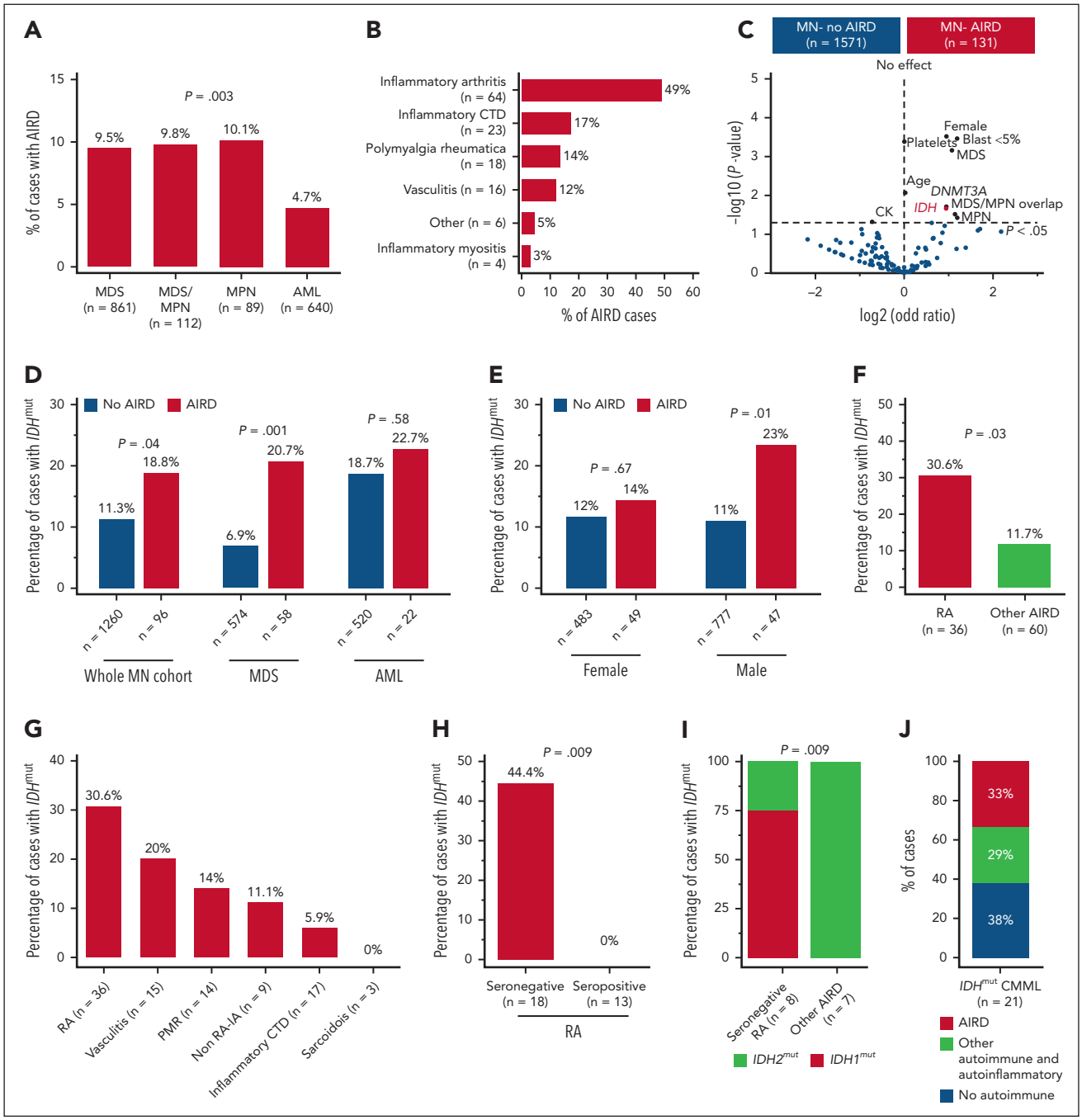

Figure 1. High prevalence of *IDH* mutation in myeloid neoplasm with RA. (A) In all, 9.5% of cases with MDS had AIRD compared with only 4.7% of cases with AML (*P* = .003). (B) Inflammatory arthritis is the most prevalent AIRD in the MN-AIRD cohort. Inflammatory arthritis includes RA plus peripheral spondyloarthropathy. (C) Volcano plot comparing the blood counts, bone marrow blast, cytogenetic changes, and somatic mutation profile of MN-AIRD vs MN without AIRD. (D) Enrichment of *IDH* mutations in MN-AIRD compared with MN without AIRD, especially MDS-AIRD compared with MDS without AIRD. (E) Unexpected enrichment of *IDH* mutation observed in male cases with MN-AIRD but not in female cases with MN-AIRD. (F) High burden of *IDH* mutation in MN with RA compared with MN with other subtype of AIRD. (G) Frequency of *IDH* mutation according to subtype of AIRD in MN. (H) Strikingly high frequency of *IDH* mutation in seronegative RA compared with seropositive RA. (I) Majority of seronegative RA harbors *IDH1* mutation, in contrast to *IDH2* mutation in other MN-AIRDs. (J) In an independent validation cohort, one-third of patients with IDH^{mut} CMML have AIRD. The χ^2 test was used to determine associations between categorical variables. CTD, connective tissue diseases.

disease onset. In 84% (n = 65) and 5% (n = 4) of cases with available information (n = 78), MN was diagnosed >12 and 1 to 12 months after the AIRD diagnosis, respectively, and in 6% (n = 5) and 5% (n = 4) of cases, MN was diagnosed within 1 month and >1 month before the AIRD diagnosis, respectively. The interval between diagnosis of RA and MN (latency) tended to be shorter in seronegative vs seropositive RA (34.6 vs 135.4 months; *P* = .06) (supplemental Figure 1F). Importantly, the interval from AIRD to MN was markedly shorter in *IDH1/2*-mutant cases compared with *IDH* wild-type cases (44.4 vs 106.3 months; *P* = .02) (Figure 2A) and even shorter in patients with *IDH1* vs *IDH2* (12.9 vs 85.6 months; *P* = .04) (Figure 2B). This short latency between AIRD and MN could be explained by the clonal expansion rate of 20% and 10% per year of *IDH1*- and *IDH2*-mutated clones, respectively, which is faster than the yearly expansion rate of 5% for *DNMT3A* and *TP53* clones.[7]

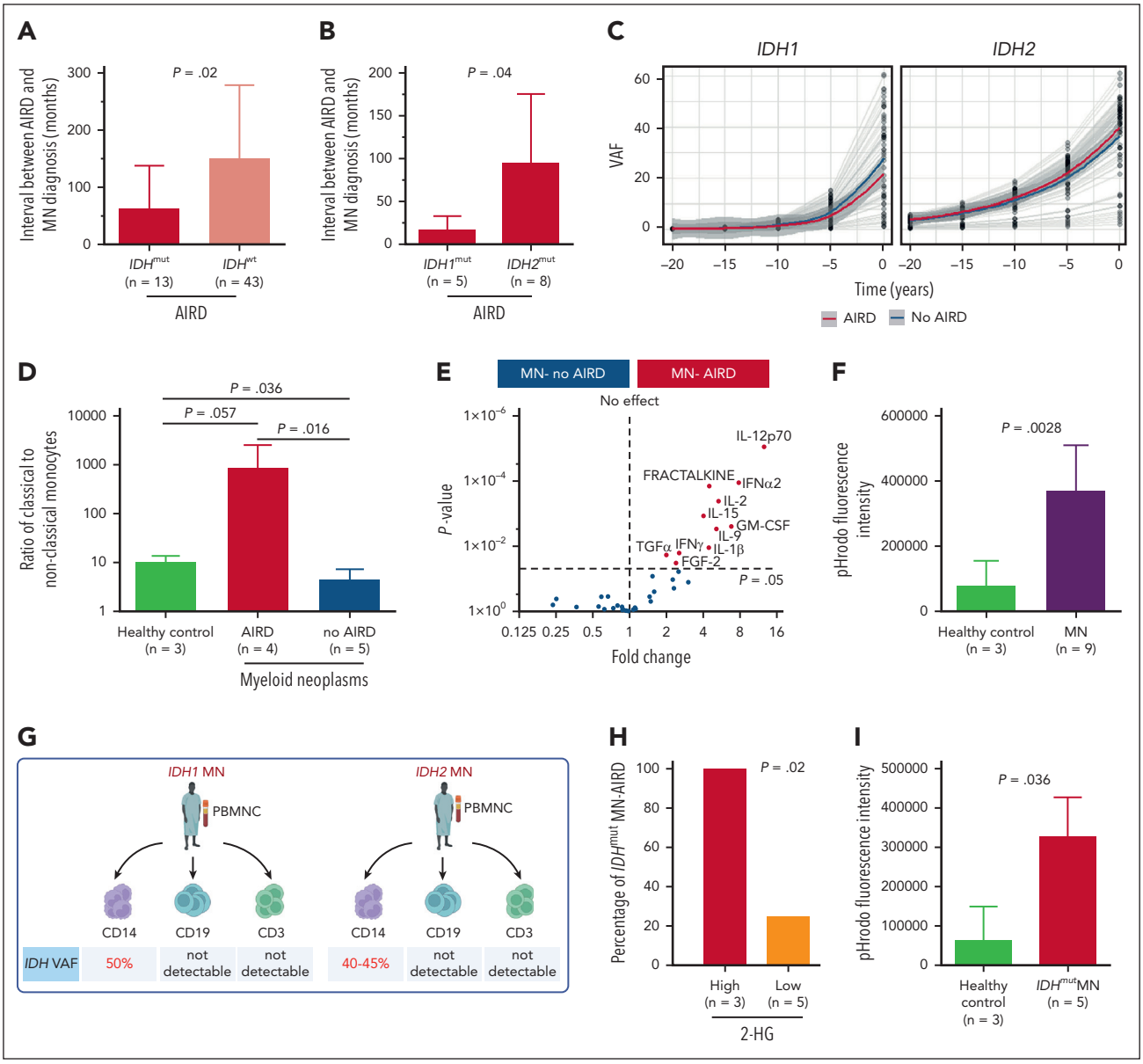

Figure 2. *IDH*-mutant clones are likely to be present prior to diagnosis of autoimmune disease. (A) Interval between AIRD and MN diagnosis was shorter in *IDH*-mutated MN-AIRD compared with *IDH* wild-type MN-AIRD particularly in (B) *IDH1*-mutated compared with *IDH2*-mutated MN-AIRD. (C) Locally estimated scatterplot smoothing (LOESS) analysis to calculate clonal expansion kinetics of IDH^{mut} and to evaluate if *IDH* clones were present at the time of AIRD diagnosis. Within each "window," a weighted average was calculated, and the sliding window passes along the x-axis. The shaded area indicates the 95% confidence interval. Gray lines represent patients. (D) Aberrantly high ratio of proinflammatory classical to nonclassical monocytes in patients with MN-AIRD (n = 4) compared with patients with MN without AIRD (n = 5) and age-matched healthy controls (n = 3). (E) Proinflammatory cytokines secreted by innate immune cells including GM-CSF, IL-12, fractalkine, IL-15, and IL-1β were significantly higher in bone marrow plasma of MN-AIRD (n = 9) compared with MN without AIRD (n = 109). (F) Aberrantly high macrophage phagocytic activity, assessed by pHrodo Red *Staphylococcus aureus* Bioparticles uptake, in subjects with MN (n = 9) compared with healthy controls (n = 3). (G) Schema of *IDH* mutational status interrogation in various cell populations ($CD14^+$ monocytes, $CD19^+$ B cells, and $CD3^+$ T cells) in 2 patients with *IDH* mutation. (H) All IDH^{mut} MN with high bone marrow plasma level of 2-HG had AIRD compared with only 25% of cases with low 2-HG level. (I) Aberrantly high in vitro macrophage activity in subject with IDH^{mut} MN (n = 5) compared with healthy control (n = 3). All bars indicate mean, and all error bars indicate SD. The Mann-Whitney test was used to detect statistically significant differences between cohorts. The χ^2 test was used to determine associations between categorical variables. FGF-2, fibroblast growth factor 2; TGFα, transforming growth factor α; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-α2, interferon α2; IFNγ, interferon γ; VAF, variant allele frequency.

Furthermore, extrapolation of the *IDH1/2*-mutant clonal burden prior to MN diagnosis indicates that the majority of clones are expected to be detectable at the time of AIRD diagnosis (Figure 2C).

AIRD are thought to be driven through adaptive immunity. However, autoinflammatory disorders such as VEXAS (vacuoles, E1 enzyme, X linked, autoinflammatory, somatic) syndrome are associated with innate immune activation and MDS.[8] We therefore analyzed T-cell subsets and monocyte and macrophage function in AIRD vs non-AIRD MN. We found a higher ratio of proinflammatory $CD14^+CD16^-$ classical monocytes to nonclassical anti-inflammatory $CD14^-CD16^+$ monocytes in MN-AIRD compared with age-matched controls and cases of MN without AIRD (Figure 2D; supplemental Figure 2). In contrast, no difference in T-cell subsets (including naive, central memory, effector memory, and terminally differentiated $CD4^+$ and $CD8^+$ T cells) was observed (supplemental Figure 3). Furthermore, we observed increased levels of proinflammatory cytokines, including granulocyte-macrophage colony-stimulating factor, interleukin-12 (IL-12), fractalkine, IL-15, and IL-1β, in the bone marrow plasma of patients with MN-AIRD vs those without AIRD (Figure 2E). Consistent with these cytokines being predominantly derived from innate immune monocyte/macrophages rather than T cells, we observed increased monocyte-derived phagocytic activity (assessed by flow cytometry) in MN (Figure 2F).

We then isolated $CD14^+$ monocytes, $CD3^+$ T cells, and $CD19^+$ B cells from 2 patients with AIRD with known *IDH1/2* mutations (Figure 2G). Interestingly, high VAF clones were detected in monocytes (40%-50%) but not in T cells (<1%) or B cells (<1%) from both patients (Figure 2G; supplemental Figure 4), consistent with intracellular (R)-2-hydroxyglutarate (2-HG) production in monocyte/macrophage cells rather than T cells, leading us to assess 2-HG levels in patients with AIRD. Strikingly, all 3 cases with high 2-HG (bone marrow metabolite peak abundance above median of $>9.3 \times 10^5$) had AIRD compared with only 1 of the 5 cases with low 2-HG ($<9.3 \times 10^5$ median abundance, $P = .02$) (Figure 2H). Finally, we noted significantly higher macrophage phagocytic activity in subjects with *IDH1/2*-mutant MN compared with controls (Figure 2I).

IDH mutations are neomorphic and lead to the production of an alternative metabolite in the citric acid cycle, 2-HG, which inhibits TET2 deoxygenase with resultant genomewide epigenetic modifications. This study demonstrates the enrichment of IDH^{mut} in MN-AIRD, in line with previous findings.[4] Importantly, our study demonstrates a strong association between *IDH*-mutated MN and a specific type of AIRD-seronegative RA, as well as a temporal relationship between *IDH*-mutant clones and AIRD. The observed elevated 2-HG in seronegative RA and *IDH1/2* mutations in myeloid rather than T cells indicates a causative association between *IDH1/2* mutation and AIRD. Emerging literature from in vitro[9] and in vivo studies reports proinflammatory effects of *IDH* mutations on monocytes[9] and macrophages,[10] in addition to an elevated IL-18 level in individuals with *IDH1* clonal hematopoiesis.[11] Collectively our findings suggest a causative relationship between *IDH1/2* mutations, MN, and seronegative AIRD, meriting further exploration of mutant IDH inhibitors such as ivosidenib,[12] enasidenib,[13] or complex I inhibitor[14] as therapeutic options for seronegative RA.

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Authorship

Contribution: L.E.H., M.D.W., and S.P. collected and annotated autoimmune rheumatic disorders, provided rheumatology expertise, and edited the manuscript; M.K. performed cytokines/chemokines profiling, helped with analysis, and edited the manuscript; A.S. performed monocytes, macrophages, and T-cells subsets analyses and edited the manuscript; K.L. and S.S. performed and analyzed 2-HG levels assay and edited the manuscript; C.T.-P. performed IDH sequencing in isolated T cells and edited the manuscript; R.C. and J.J.L. collated clinical data of myeloid neoplasms and autoimmune rheumatic diseases and edited the manuscript; H.S.S., A.B., S.B., R.J.D., S.E.S., and C.N.H. profiled somatic mutation of the samples and edited the manuscript; D.T.Y. edited the manuscript; N.R. provided expertise in macrophage function and edited the manuscript; R.T. provided expertise on autoimmune rheumatic disease and edited the manuscript; M.P. contributed validation cohort and edited the manuscript; D.T. edited the manuscript, designed experiments, and supervised mass spectrometry analyses and IDH sequencing in isolated T cells; C.H.K. performed statistical analysis and edited the manuscript; M.V.S. contributed the patient data and edited the manuscript; and D.K.H. designed the project, secure funding, supervised, analyzed results, and wrote the manuscript.

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Footnotes

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Additional methods and data can be found in the supplementary Data. For original data, please contact corresponding author, Devendra K. Hiwase (devendra.hiwase@sa.gov.au).

The online version of this article contains a data supplement.

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