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Frequent reconstitution of IDH2^{R140Q} mutant clonal multilineage hematopoiesis following chemotherapy for acute myeloid leukemia

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While the full repertoire of recurrent genetic lesions associated with acute myeloid leukemia (AML) is now likely known, the pathogenic contribution of each remains incompletely understood. Some are compatible with otherwise normal hematopoiesis. For example, aging humans with normal blood counts often exhibit hematopoietic sub-clones with mutations in *DNMT3A*, *ASXL1* or *TET2*, among others, even though only a small minority ever develops myeloid malignancy.1–3 This suggests that such genetic lesions confer a competitive advantage to hematopoietic stem and progenitor cells (HSPC) to facilitate formation of a pre-leukemic stem cell pool receptive to secondary mutations for full-fledged leukemic transformation.4 Consistent with this, *DNMT3A* mutations often persist in blood many years after successful chemotherapy for AML.5 Conversely, other genetic lesions appear tightly associated with malignant transformation. For example, mutations in *NPM1* are not seen with aging and their persistence following chemotherapy predicts for impending relapse.6

Mutations in *IDH1* and *IDH2* are found in 15-20% of patients with AML7 but are extremely rare drivers of clonal hematopoiesis in aging (~100-fold less frequent than mutations in *DNMT3A*, for example).1–3 It is currently unclear whether *IDH* mutant (*IDH*^{mut}) clones persist in long-term clinical remission, and if so, what is their natural history, clinical fate,

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multilineage differentiation potential and relationship to other mutations. Establishing this is essential not only for biologic understanding, but also to establish therapeutic endpoints in patients treated with mutant IDH inhibitors.

To address this we studied 23 patients presenting with AML or refractory anemia with excess blasts (RAEB) associated with mutations in *IDH1* (n=10) or *IDH2* (n=13) (Table S1), all of whom achieved complete morphologic remission (CR) after one or two courses of induction chemotherapy. In each case there was reduction in *IDH* mutant allele frequency (MAF) by quantitative digital PCR (dPCR) following treatment.8 However, samples collected at CR1 (after course 1 (n=6) or 2 (n=17)) demonstrated that only 3/23 (13%) had an undetectable *IDH* mutation burden (sensitivity ≤0.1% MAF) (Figure 1a). Following consolidation chemotherapy *IDH* MAF variably decreased or increased (Figure 1b). Considering all patients at completion of chemotherapy, only 5/23 (22%) had undetectable IDH^{mut} alleles (Figure 1c). Thus IDH^{mut} clones typically persist at the end of induction chemotherapy in the majority of patients achieving morphologic CR and are not eradicated by consolidation chemotherapy.

By contrast, of seven patients receiving hematopoietic stem cell transplant (HSCT) (including one transplanted in CR2), IDH^{mut} alleles were undetectable in bone marrow (BM) in five (71%) by day 100 (Figure 1d). Of these, three survive with undetectable IDH^{mut} alleles 15, 22 and 51 months post-transplant. One died in CR1 14 months post-transplant of procedure-related complications with undetectable $IDH1^{R132C}$, and the fifth suffered graft failure, low-level reconstitution of $IDH1^{R132C}$-mutant hematopoiesis and died 22 months post-transplant. Of the two others, one died of complications early post-transplant and the other failed to clear $IDH2^{R172K}$ following HSCT, heralding early relapse. Thus, in contrast to chemotherapy, allogeneic HSCT is effective in durable eradication of IDH^{mut} hematopoiesis.

We next evaluated outcomes of 16 patients treated with chemotherapy alone for whom more than six months' follow up was available (including one subsequently transplanted in CR2). Three of six $IDH1^{mut}$ patients relapsed following a progressive rise in MAF, whereas three exhibit persistent low level *IDH1* mutant clones (<1% MAF) and remain in CR1 after 7, 22 and 24 months' follow up respectively (Figure S1). Four of 10 $IDH2^{mut}$ patients also relapsed, again following a progressive rise in MAF (Figure 1e). In contrast, six $IDH2^{mut}$ patients (all with $IDH2^{R140Q}$ mutations) remain in CR after 9-52 months' follow up with normal or near-normal blood counts (Table S2) despite persistent and substantial $IDH2^{R140Q}$ mutant clones, which in some cases dominate hematopoiesis (Figure 1f). As expected, plasma D-2-hydroxyglutarate (D-2-HG) levels mirrored MAFs during CR (Figure 1g).8 Thus, $IDH2^{R140Q}$ mutant hematopoietic clones frequently persist and may predominate in patients with normal or near-normal hematopoiesis following successful chemotherapy for AML.

We next addressed the hierarchical placement of *IDH* mutations versus other known drivers in myeloid cancer using targeted next-generation sequencing in the 23 presentation samples (Table S1). We identified 109 high confidence somatic variants in 26 genes (median 4 per sample; range 2–9; Figure S2; Table S3) and confirmed the presence of all IDH mutations,

with concordant MAFs versus dPCR (Figure S4). The *IDH*^{mut} clone was dominant in 12/23, a major sub-clone in 7/23 and a minor sub-clone in 4/23 cases (Figures 1h, 1i, S4, S5, Table S3). Where dominant, the *IDH* MAF was similar to that of co-occurring mutations in 8/12 cases precluding identification of the ancestral mutation. However, in 4/12 cases (BB93, BB161, BB187 and BB484) the *IDH* MAF was larger, suggesting that mutations in *IDH1* or *IDH2* can predate mutations in *SRSF2*, *JAK2* and *DNMT3A*. In 11 cases where the *IDH*^{mut} clone was sub-clonal, the dominant clone exhibited mutations in *DNMT3A* (n=6), *SRSF2* (n=5) or *STAG2* (n=1), indicating that in these instances mutations in IDH1 or IDH2 were secondary events. Sub-clonal placement of IDH mutations had no clear impact on the likelihood of persistence post-chemotherapy.

Sequencing of remission samples identified persistence in CR1 of mutations in *DNMT3A*, *RUNX1, JAK2*, *ASXL1* and *RAD21*, in addition to those in *IDH1* and *IDH2*. *NPM1* and *FLT3*-ITD mutations were not detected in remission samples (Figures S4, S5, Table S3). Illustrative of this, five IDH2^{R140Q} mutant patients who remain in long-term CR presented with a concomitant *NPM1* mutation (Table S3). In each case the *NPM1* mutation has remained undetectable despite persistence of the *IDH2*^{mut} clone (Figures 2a-c, S6), which exhibited additional persisting concomitant mutations in two patients (in *SRSF2*, *BCOR* and *GATA2*; Figures 2c, S6). By contrast, relapsing *IDH*^{mut} patients exhibited recrudescence of mutations in *NPM1* (BB120, BB475) or *RUNX1* (BB85, BB235, BB350) as determined by Sanger sequencing or digital PCR (data not shown). Thus mutations in *SRSF2* and *DNMT3A* may precede or follow those in *IDH1* or *IDH2*, whereas *FLT3*-ITD or mutations in *NPM1* appear secondary and associated with the presence of frank leukemia.

To determine whether post-chemotherapy IDH2^{R140Q} clones contribute to multilineage hematopoiesis, we isolated by flow-sorting phenotypically defined high purity cell populations from the BM and blood of two individuals in prolonged CR (Figure S7). In both patients neutrophils and monocytes were predominantly derived from IDH2^{R140Q} mutant clones, as was BM erythropoiesis and myelopoiesis (Figures 2a,b). The B-lineage was derived from an IDH2^{R140Q} clone predominantly in BB287 and to a lesser extent in BB161. The T-lineage was not involved. The immunophenotypic BM hematopoietic stem cell (HSC) compartment exhibited 20-35% involvement with IDH2^{R140Q} mutant cells, with higher proportionate involvement of the downstream multipotent progenitor compartment in both cases. To interrogate further their functional potential, CD34^{+} HSPCs from deep remission time points from both patients were cultured in methylcellulose clonogenic assays. In keeping with our *in vivo* data, 96% and 50% respectively of individually isolated colonies (including both erythroid and granulocyte/macrophage colonies) were *IDH2*-mutated (Figures 2d,e), as were 60% of colonies derived from single flow-sorted immunophenotypic HSCs from BB287 (Figure 2f). Similar analyses in a patient with persistent *IDH1*^{mut} hematopoiesis (BB355) 22 months after presentation demonstrated involvement of neutrophils and monocytes, but not lymphocytes (Figure S8).

In particular, our data demonstrate dynamic reconstitution of IDH2^{R140Q}-mutated, functionally normal, clonal hematopoiesis following successful chemotherapy for AML. Such clones may on occasion exhibit a concomitant SRSF2^{P95R} mutation. Our observations provide compelling *in vivo* evidence for the pre-leukemic nature of IDH2^{R140Q} mutations in

view of their multilineage differentiation potential. These data extend insight into recent observations that immunophenotypic HSCs carry *IDH* mutations at diagnosis in 80% of IDH^{mut} AMLs,9 whilst xenografts from 36% of IDH^{mut} AML patient samples generated non-leukemic multilineage grafts.10

One unique feature of IDH^{mut} AML is the generation of D-2-HG. This metabolite was present at high levels in the plasma of patients with persistent IDH^{mut} clonal hematopoiesis (Figure 1g), an observation which counters the concept that D-2-HG is directly transforming, as has recently been suggested.11 Nevertheless, caution is mandated for the long-term outcome of these patients which to date remains unclear.

Importantly, our findings indicate that approaches tracking *IDH* mutations (or D-2-HG as their surrogate) will not reliably detect minimal residual leukemia, in contrast to recent claims.12,13 Further, total eradication of IDH^{mut} clones (in particular $IDH2^{R140Q}$-mutant clones) as a therapeutic goal, for example using specific pharmacological inhibitors, while desirable, may not be necessary to achieve prolonged disease-free survival given that IDH mutations and leukemic disease are not tightly linked in many cases. That said, it remains unclear whether our conclusions extend to patients with $IDH1^{R132}$ or $IDH2^{R172}$ mutations because in our cohort persistence or recurrence usually heralded leukemic relapse, and the three cases with persistent $IDH1^{mut}$ hematopoiesis display low MAFs. This hints at discrepant biology associated with different IDH mutations, in keeping with reported biochemical differences.14 More broadly, our findings add $IDH2^{R140Q}$ to *DNMT3A*, *TET2* and other mutations as mediators of clonal hematopoiesis of indeterminate potential,15 at least after chemotherapy for established AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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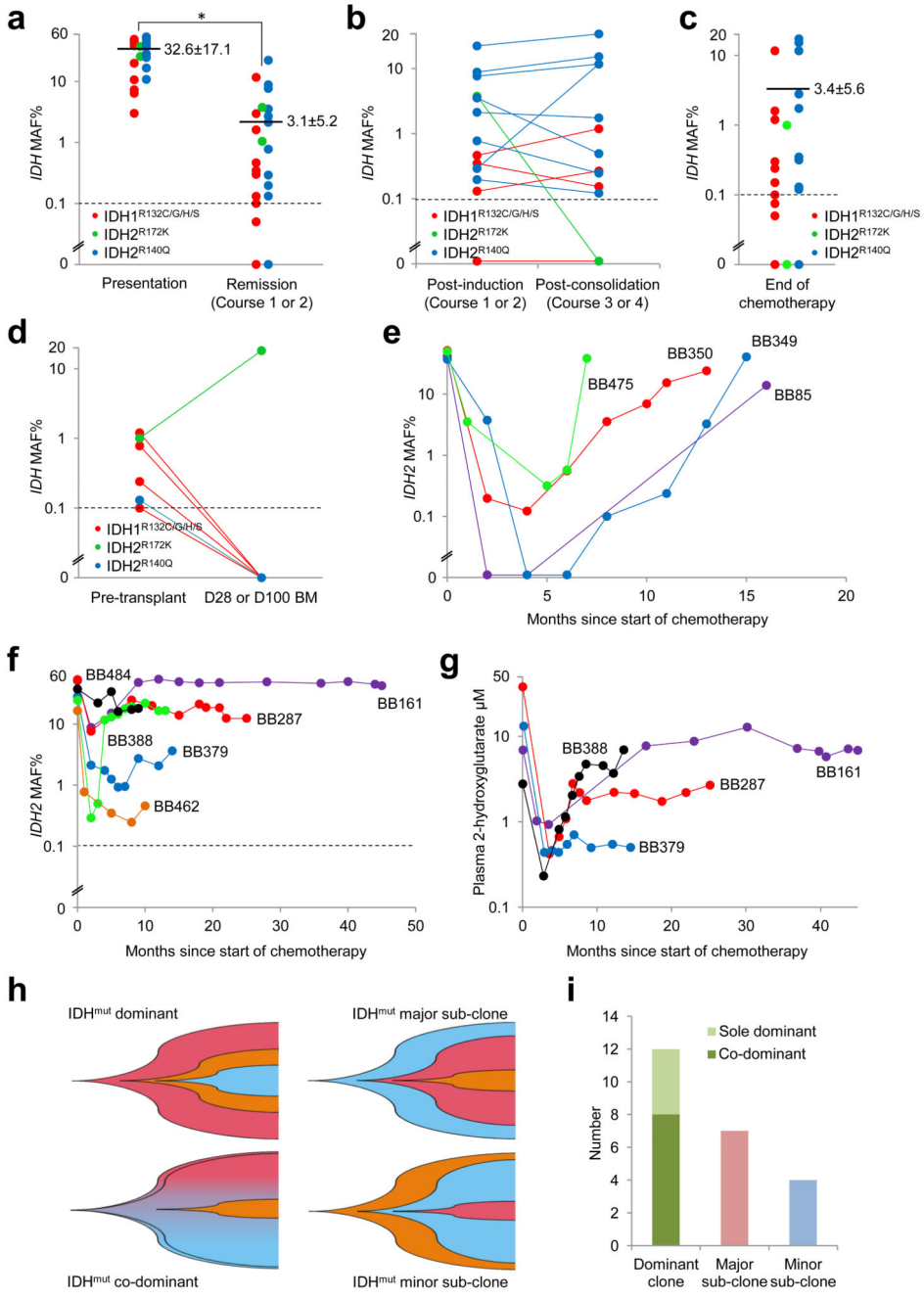

Figure 1. *IDH* mutant allele frequencies in AML/RAEB patients following treatment.

(a) *IDH* MAFs at presentation and at complete morphologic remission (samples collected after course 1 (n=6) or 2 (n=17)). * indicates p<0.001 by paired t-test. Mean±SD MAFs are shown. (b) *IDH* MAFs post-induction (i.e. after course 2) and post-consolidation in patients receiving one or more cycles of consolidation chemotherapy (n=13). (c) *IDH* MAFs at completion of intensive chemotherapy in patients treated with chemotherapy alone (n=16) or immediately pre-transplant in those allografted (n=7). (d) *IDH* MAFs pre- and post-transplant (day 28, n=1, day 100, n=5). (e) BM *IDH2* MAFs in four patients who relapsed

following chemotherapy. Morphologic relapse was documented at the last time point shown. (f) BM *IDH2* MAFs in six patients in sustained morphologic CR. Biobank identifiers are shown. (g) Total plasma 2-hydroxyglutarate levels in four patients from (f). (h & i) Hierarchical placement of IDH mutations, as determined by targeted next generation sequencing. Fish plots (h) illustrate exemplar patterns of IDH^{mut} acquisition. IDH^{mut} clonal hematopoiesis is shown in red with hypothetical mutations X and Y shown in blue and orange respectively. (i) Graph shows categorization of types of IDH^{mut} clones in 23 presentation samples. An IDH^{mut} clone was considered dominant if the *IDH* MAF was the highest of all detected mutations. Where the *IDH* MAF was proportionately 15% larger than that of any other mutation the clone was considered sole dominant; otherwise it was deemed co-dominant. Where the *IDH* MAF was 50-85% of that of the highest mutation, the IDH^{mut} clone was considered a major sub-clone; otherwise it was deemed a minor sub-clone.

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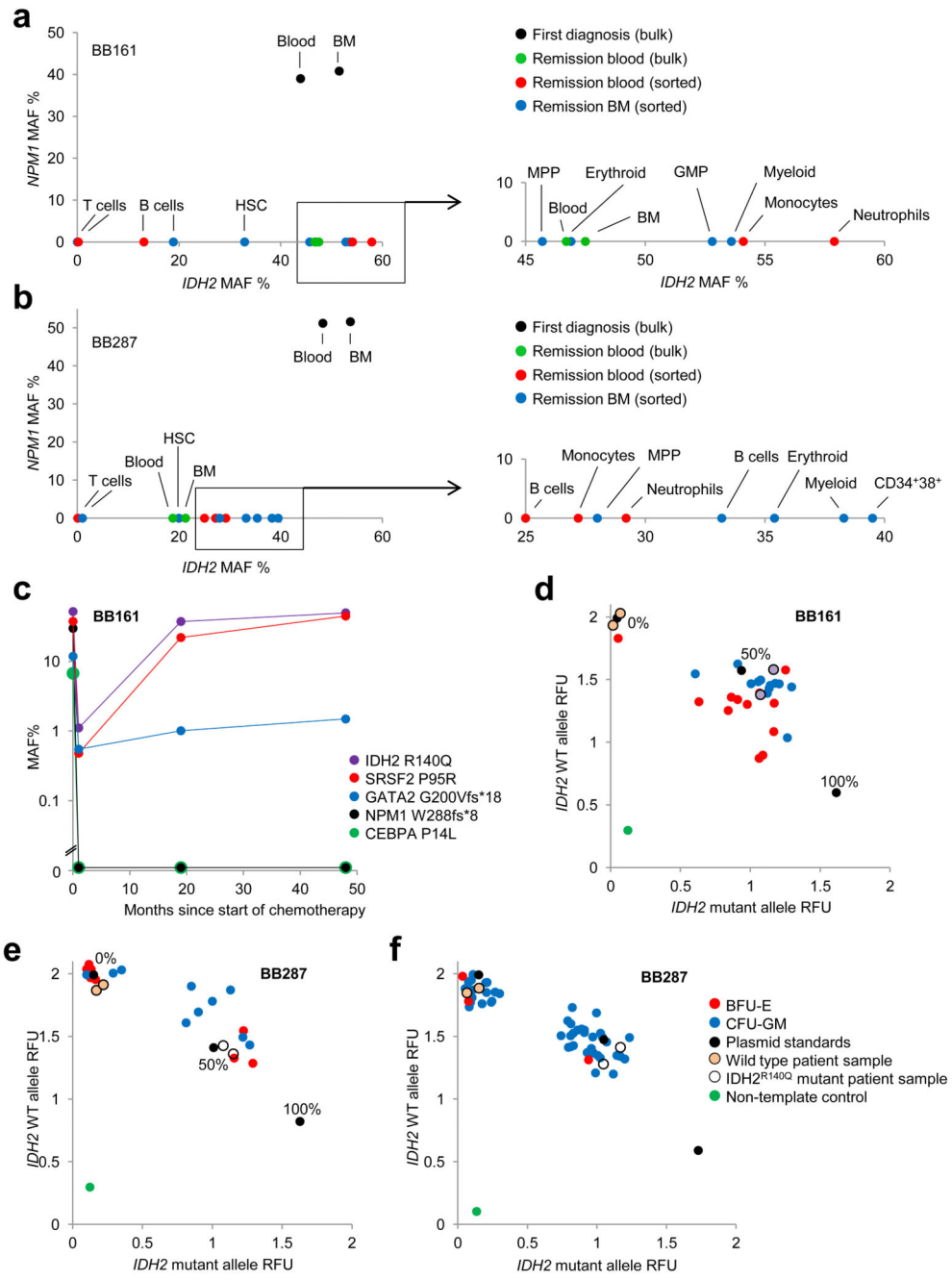

Figure 2. Multilineage contribution of IDH2^{R140Q} mutant clonal hematopoiesis.

Graphs show *IDH2*^{R140Q} versus *NPM1* MAFs at the indicated time points and in the indicated cell populations in (a) BB161 and (b) BB287. Remission samples were collected 20 and 18 months following presentation, respectively. (c) MAFs for the somatic mutations identified by targeted next generation sequencing at the indicated time points following presentation in patient BB161. (d) and (e) show allelic discrimination dPCR plots for plucked single colonies isolated following 14 days of culture of CD34^{+} BM HSPC collected 40 months (BB161) and 18 months following presentation (BB287). Plasmid standards

containing 0%, 50% and 100% mixes of DNA sequences coding for $IDH2^{R140Q}$ & $IDH2^{WT}$ are shown. Control samples from $IDH2^{WT}$ and $IDH2^{R140Q}$ mutated bulk patient samples are also shown (n=2 for each). (f) Allelic discrimination dPCR plot for individual colonies derived from single-sorted phenotypic HSCs ($CD34^{+}38^{-}90^{+}45RA^{-}Lin^{-}$) cultured in individual wells in methylcellulose for 14 days from BB287 17 months following presentation. BFU-E, burst forming unit erythroid; CFU-GM – granulocyte/macrophage colony forming unit; RFU, relative fluorescent units.

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