inversely correlated with their intrinsic GTPase activity. Ruess et al.[3] used a KRAS-G12D-specific antibody to infer that SHP2 inhibition selectively modified the levels of active KRAS-G12D. These studies suggest that KRAS oncoproteins undergo nucleotide cycling in cancer cells, which in turn makes them sensitive to nucleotide exchange and susceptible to inhibition of SHP2 (and possibly susceptible to the inhibition of other factors that regulate nucleotide exchange). This is consistent with earlier findings[6,12] showing that KRAS-G12C undergoes nucleotide cycling in cancer cells, which is why allele-specific inhibitors suppress KRAS-G12C–GTP levels even though these inhibitors bind to the inactive state of the oncoprotein.

More work is needed to understand exactly how SHP2 regulates KRAS–GTP. If this occurs through the phosphatase activity of SHP2, then which are the key substrates involved? Alternatively, if it's through its role as a scaffold, are proteins other than SOS1 involved? These questions aside, the studies by Algül, Bass and Bernards and their colleagues[2–4] make important strides in our effort to better understand and to therapeutically target KRAS-driven cancers. As it stands, clinical efforts are directed at determining the therapeutic potential of SHP2 inhibitors in patients with RTK-driven tumors. The studies in this issue make a very compelling argument that SHP2 inhibitors, in combination with MEK inhibitors, ought to be tested in patients with KRAS-mutant lung or pancreatic adenocarcinoma and those with *KRAS*-amplified gastric carcinoma. ❐

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Competing interests

The authors declare no competing interests.

CANCER GENOMICS

Predicting progression to AML

The number, identity, and burden of mutations in clonal hematopoiesis are associated with the risk and timing of progression to acute myeloid leukemia.

Rob S. Sellar, Siddhartha Jaiswal and Benjamin L. Ebert

Acute myeloid leukemia (AML) is an aggressive malignancy characterized by clonal expansion of undifferentiated myeloid precursors that results in impaired hematopoiesis and bone marrow failure. Recent studies of clonal hematopoiesis, an expansion of blood cells derived from a single hematopoietic stem cell, indicate that for some individuals, clonal hematopoiesis is a premalignant state that may be identified many years before the development of AML or another hematological malignancy. Because of increasingly sensitive methods of detection, it may be possible to identify clonal hematopoiesis within all individuals as time progresses[1]. Therefore, it is important to identify clonal hematopoiesis that is of clinical significance rather than a potentially benign aspect of aging.

Clonal hematopoiesis of indeterminate potential (CHIP) can be found in otherwise healthy adults[2]. Large prospective cohort studies with well-annotated health outcomes and in which whole-exomes sequencing (WES) of banked blood cells was performed have shown conclusively that CHIP is associated with increased mortality[3,4], which is predominately driven by cardiovascular disease[4,5]. However, it is the risk of developing a hematologic malignancy that is most dramatically elevated. This risk of hematological malignancy is not the same for all patients with CHIP and increases with both the clone size and the number of mutations (clonal complexity)[3,4]. However, initial studies were not powered to find associations with specific genes or with the type of hematologic malignancy.

Two new studies—one in *Nature*[6] and one in this issue of *Nature Medicine*[7]—further investigate the progression of clonal hematopoiesis to hematologic malignancy but focus specifically on the development of AML (Fig. 1). Both of these studies took advantage of large population-based cohorts in which blood cell DNA was prospectively banked before the onset of disease and therefore were able to analyze premalignant tissue. Both studies also utilized a nested case-control study design in which individuals from the cohort with a relatively rare outcome, in this case AML, were identified and drawn from larger population studies rather than using an unselected population. This method provides higher power to detect associations and estimate risk effects at smaller sample sizes. Furthermore, both studies carried out targeted sequencing (rather than WES) on genes known to be involved in leukemias, which provided the advantage of greater sequencing depth and sensitivity but with diminished breadth of coverage.

Abelson et al.[6] identified 95 patients with AML from a previously studied prospective European population cohort of >500,000 individuals from whom samples were collected on average 6.3 years before a diagnosis of AML (pre-AML). From this same cohort, they studied 414 unaffected individuals matched to the affected individuals for age and gender and a smaller validation set that included affected and unaffected individuals. The authors found that in the pre-AML group, clonal hematopoiesis with a putative driver mutation was more common than

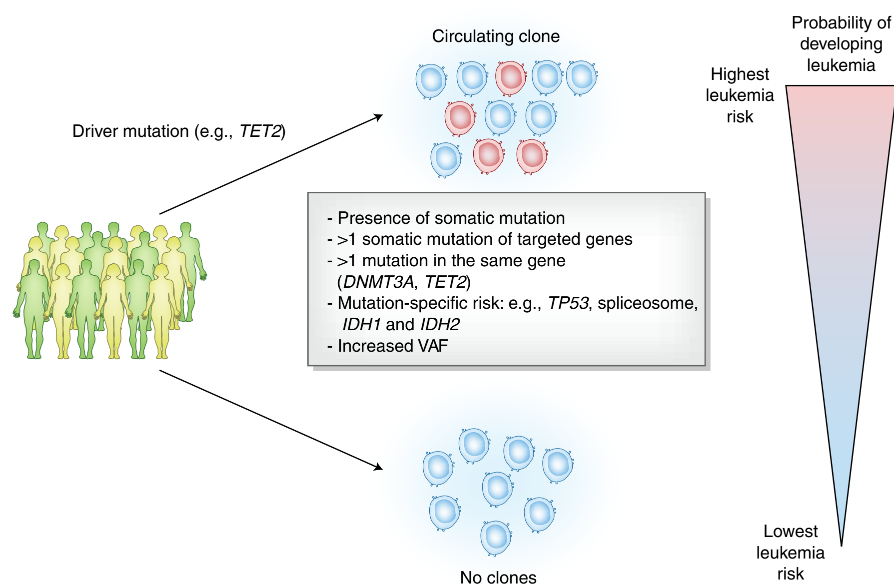

Fig. 1 | The risk of developing AML. Using targeted sequencing in nested case-control studies, Abelson et al.[6] and Desai et al.[7] identify features of clonal hematopoesis that predict progression and/or timing of progression to AML. Key features of high-risk AML include the presence of a somatic mutation (indicated as a red cell), the number of mutations, the presence of some higher-risk mutations, and a higher VAF. Abelson et al.[6] also identified clinical features that were able to predict risk.

in unaffected individuals (73.4% versus 36.7%). This group also demonstrated that the subsequent development of AML was associated with an increase in both the clone size and median number of mutations per individual, including those in *TP53*, one of the most commonly genes mutated in cancer, and *U2AF1*, a key gene in the regulation of splicing.

An association of higher red cell distribution width (RDW) with CHIP and excess mortality has been identified previously[4]. After investigating a number of clinical parameters, Abelson et al.[6] also found that higher RDW was associated with an increased risk of progression to AML, an association that was verified in an additional 875 cases. They devised a model using information from blood counts that could predict the development of AML at 6–12 months before diagnosis. Thus, the use of routinely available clinical features could predict the development of AML, albeit with less forewarning than the genetic model and with limited sensitivity (26%).

Desai et al.[7] assessed participants in the Women's Health Initiative, a prospective study of over 160,000 women in the United States, of whom 212 developed AML. Mutations in *DNMT3A*, *TET2*, *JAK2*, genes encoding spliceosome components, and *TP53*, among the most common mutations in both CHIP and hematological malignancies, were significantly more common in individuals that subsequently developed AML.

In this cohort, having a mutation in one of the targeted genes at a median of 9.6 years before the diagnosis of AML was associated with an increased risk of developing AML relative to the healthy people in the cohort, and this risk was independent of age. As in the study by Abelson et al.[6], the authors of this study observed a lower risk of progression to AML in those with somatic mutations in targeted genes compared with progression to AML and/or hematological malignancy in those with mutations as identified by studies using WES[3,4]. This is possibly because targeted sequencing has increased sensitivity and will detect more low-level mutations in a healthy population, thus lowering the observed relative risk. Indeed, if one assesses risk of progression in the investigations by these authors using the larger clone-size cutoff described in prior studies, then the relative risk is in fact very similar to those previous reports[3,4]. Increased progression to AML was seen for those with >1 mutated gene by targeted sequencing (increased complexity) and ≥10% variant-allele fraction (VAF; increased clone size), although the impact of VAF was not seen for those with *TP53* or *IDH1* and *IDH2* (*IDH1/2*) mutations, as all individuals developed AML.

Although the findings of both of the studies are generally concordant, there were some differences. Despite their prevalence in myeloid malignancies and CHIP, mutations in additional sex combs-like 1 (*ASXL1*) were only enriched in those who progressed to AML in the Abelson cohort[6]. In addition, in contrast to the study by Abelson et al.[6], in which *IDH1/2* were individually of moderate to low risk, Desai et al.[7] found that *IDH1/2* mutations assessed in combination were of particularly high risk for transformation. This difference may reflect the importance of later cooperating mutations in, for example, nucleophosmin 1 (*NPM1*) that were not identified at baseline in either study and that were enriched in both AML with *IDH* mutations and AML in females[8]. As neither study focused on genetic analysis of AML at the time of diagnosis, these questions remain to be addressed.

Much of the predictive power of these genetic models comes from the size and complexity of the identified clones and is therefore in agreement with the known data for hematologic malignancy as a whole. A key additional aspect of these studies is the identification of a small number of specific mutations or combinations of mutations that appear to have prognostic power in the context of AML in addition to the size of the hematological clone and mutation burden. One limitation of these studies is, given the case-control study design, the inability to obtain absolute, as opposed to relative, risk estimates for mutations. Nonetheless, the new predictive model using RDW and routinely available clinical data is an encouraging proof of principle that individuals at greatest risk can be identified up to a year before diagnosis. For a relatively infrequent malignancy such as AML, such a model would need to have improved sensitivity to be clinically useful. However, as this would likely increase the number of false positives, identified individuals could then be the focus of targeted sequencing in an attempt to maintain specificity.

The challenge for the field is therefore to build on these studies and design such integrated clinical and molecular models with high sensitivity and specificity that can be validated in larger prospective analyses, ideally looking beyond AML and assessing the multiple sequelae of CHIP, including other malignancies and cardiovascular disease. Perhaps most critical is the need to identify interventions that can be offered to those found to be at highest risk. In contrast to the availability of surgical intervention in the premalignant states of solid tumors, it is not possible to surgically eliminate clonal hematopoiesis. However, there are established and/or emerging pharmacologic therapies for mutations in *JAK2*, *IDH1/2*

(ref. [9]), and genes encoding spliceosome components[10], as well as recent preclinical advances in restoring *TET2* function[11,12]. The ultimate aim of such integrated models would be to test interventions that may mitigate the adverse consequences of CHIP. These new reports provide another useful step toward such models and the rational design of interventional studies. ❐

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Competing interests

The authors declare no competing interests.

MICROBIOME

Microbiome metabolomics reveals new drivers of human liver steatosis

An integrative multiomics approach in nondiabetic obese women identifies phenylacetate as a microbial metabolite contributing to the accumulation of lipids in the liver and hence to nonalcoholic steatohepatitis.

Nathalie M. Delzenne and Laure B. Bindels

Up to a quarter of patients with nonalcoholic fatty liver disease (NAFLD) develop a progressive inflammatory liver disease termed nonalcoholic steatohepatitis (NASH) that may progress toward cirrhosis and hepatocellular carcinoma. However, the biological events driving the progression of NAFLD are not clearly elucidated. The gut microbiota—that is, the microorganisms living in the intestine—is able to modulate host metabolism and immunity, mainly through the release of metabolites and bioactive components[1], and clearly contributes to the metabolic products in blood plasma—the plasma metabolome. Although tremendous efforts have recently been dedicated to the identification of bacterial by-products[2], the mechanism behind the beneficial or detrimental effects of key microbial metabolites on host health in the field of NAFLD remains to be studied[3]. In this issue, Hoyles et al.[4] explored the plasma and urinary metabolome of nondiabetic obese women and integrated these data with fecal metagenomics and hepatic transcriptome data to unravel the molecular pathways linking the gut microbiota to hepatic steatosis.

The authors focused on a cohort of 56 morbidly obese, weight-stable, nondiabetic women recruited in Italy and Spain who did not receive any hypoglycemic drug treatment (among numerous exclusion criteria). Across the cohort, the women presented variable levels of steatosis. This homogenous cohort allowed the authors to identify confounding factors to the analyses that were then taken into account and to focus on potential key explanatory factors for variability in the progression to steatosis. They found that the degree of steatosis and also markers of liver dysfunction were negatively associated with microbial gene richness and were positively associated with levels of Proteobacteria, Actinobacteria and Verrucomicrobia at the phyla level in the gut.

With regards to metabolite association with steatosis, the authors' characterization of microbial functions revealed, among other findings, a positive association of hepatic steatosis with gut microbial amino acid metabolism. Accordingly, the authors' fecal metabolome analysis uncovered a link between elevated branched chain and aromatic amino acid level and steatosis, as well as an increase in phenylacetate that strongly correlated with steatosis. Phenylacetate is the main phenolic compound found in fecal samples and can be derived from plant secondary compounds as well as from microbial fermentation of aromatic amino acids (mainly phenylalanine)[5]. Interestingly, *Bacteroides* spp. is likely the major contributor to microbial phenylacetate production in humans[5]. In the mammalian host, phenylacetate production from phenylalanine through hepatic transamination is normally low (except in the case of phenylketonuria), so it can be assumed that the level seen by these authors is from microbial metabolism (Fig. 1).

Then, the authors transferred the fecal material from obese women to antibiotic-pretreated mice and observed an increased accumulation of lipids in the livers of mice transplanted with the gut microbiota from the patients with grade 3 NASH as compared to mice transplanted with the gut microbiota from those with grade 0 disease. This clarified the causal role played by the gut microbiota of patients with steatosis in hepatic lipid accumulation.

In a second set of experiments, the authors fed mice a diet including phenylacetate, and this lead to increased hepatic lipid content. The authors further corroborated the causal effect of phenylacetate on steatosis in in vitro experiments in primary human hepatocytes. By doing so, the authors established that production of phenylacetate is a key component in the mechanisms through which the gut microbiota contributes to hepatic steatosis. However, as pointed out by the authors, phenylacetate administration did not fully recapitulate the full steatosis