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Haploinsufficient Transcription Factors in Myeloid Neoplasms

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Abstract

Many transcription factors (TFs) function as tumor suppressor genes with heterozygous phenotypes, yet haploinsufficiency generally has an underappreciated role in neoplasia. This is no less true in myeloid cells, which are normally regulated by a delicately balanced and interconnected transcriptional network. Detailed understanding of TF dose in this circuitry sheds light on the leukemic transcriptome. In this review, we discuss the emerging features of haploinsufficient transcription factors (HITFs). We posit that: (*a*) monoallelic and biallelic losses can have distinct cellular outcomes; (*b*) the activity of a TF exists in a greater range than the traditional Mendelian genetic doses; and (*c*) how a TF is deleted or mutated impacts the cellular phenotype. The net effect of a HITF is a myeloid differentiation block and increased intercellular heterogeneity in the course of myeloid neoplasia.

It takes two to make a thing go right.

—Rob Base and DJ E-Z Rock, "It Takes Two"

Haploinsufficient:
a dominant phenotype in a diploid organism resulting from a loss-of-function event of only one allele

Loss of function:
a mutation in which the mutant allele lacks some function of the wild-type allele

Genetic constraint:
the degree to which DNA sequence is evolutionarily conserved and variations are selected against

Triplosensitive:
a dominant phenotype arising from the additional copy of a gene in a diploid organism

Dose-sensitive: a trait arising from the gain or loss of expression of a gene

1. INTRODUCTION

By the classical genetic definition, malignancy was initially thought to be a dominant phenotype. Malignant cells fused with nonmalignant counterparts produced malignant hybrids (1). Subsequent work demonstrated that in some combinations the reverse is true; that is, fusions result in nonmalignant hybrids, creating the paradigm of a recessive mechanism of tumor suppression (2). This paradigm was formalized with the discovery of the first tumor suppressor gene, *RB1*. As described in Knudson's two-hit hypothesis, both copies of a given tumor suppressor gene must be inactivated to cause cancer (3). Nearly 30 years later, this hypothesis was challenged by the finding that loss of only one copy of P27Kip (*CDKN1B*) is sufficient to promote the development of tumors (4). P27Kip thus founded the growing class of genes that act as haploinsufficient tumor suppressor genes and implicated the dose of tumor suppressors in oncogenesis. As discussed below, extensive tumor sequencing suggests that Knudson's hypothesis may in fact apply only to a minority of tumor suppressor genes.

2. THE PARADIGM OF HAPLOINSUFFICIENCY

The classical definition of haploinsufficiency is the production of a dominant phenotype in a diploid organism after one allele of a gene undergoes a loss-of-function event (5). The context of the phenotype measured is critical and nuanced, as demonstrated in yeast. In genome-wide proliferation screens, the fraction of haploinsufficient yeast genes increases from 3% in nutrient-replete media to 20% in nutrient-deplete media. When morphological traits are also assessed, 33–59% of genes produce haploinsufficient phenotypes (6, 7). The extent of haploinsufficiency is harder to quantify in mammals; however, efforts to systematically knock out and phenotype all mouse genes reveal that a striking 42% of genes have significant phenotypes in heterozygous mice (8).

Surveys of healthy human genomes have revealed which human genes are among the most evolutionarily constrained, defined as genes that are the most sensitive to deleterious mutations. Almost half of coding genes have fewer loss-of-function variants than would be expected by chance, indicating a high degree of genetic constraint genome wide. Among constrained genes, haploinsufficient genes are the most conserved, as a deleterious variant in one allele is selected against (9). Transcription factors (TFs) appear more likely to produce haploinsufficient phenotypes when compared with other genes, and homeodomain-containing TFs are overrepresented among haploinsufficient transcription factors (HITFs) (10). Indicating the importance of finely tuned protein dose, genes predicted to produce haploinsufficient phenotypes are also likely to be triplosensitive, in that gain of an additional copy of the gene produces a dominant phenotype (11). However, measures characterizing the extent of haploinsufficiency genome wide in healthy individuals do not capture genes with deleterious alleles that do not come into play until after reproduction (e.g., *BRCA1*). Overall, these studies demonstrate the profound dose-sensitive nature of the human genome.

Just as unbiased sequencing studies have demonstrated greater haploinsufficiency across the genome than previously appreciated, similar sequencing studies of tumors highlight the inadequacy of the two-hit model of tumor suppressor genes. To measure haploinsufficient genes in cancer, Elledge and colleagues (12) performed a sophisticated pan-cancer analysis for patterns of loss-of-function mutations and gene deletions. They estimate that at least 30% of all genes exhibit haploinsufficiency. Notably, almost all sporadic tumor suppressor genes are predicted to produce haploinsufficient phenotypes. The correlation between haploinsufficiency and triplosensitivity is also observed in tumor suppressor genes. Overall, haploinsufficiency in cancer is much

more prevalent than previously appreciated. Furthermore, there is genetic pressure, even in cancer, to maintain dose-sensitive genes within a minimum and maximum range.

To highlight cardinal features of haploinsufficient genes in cancer, we turn to PTEN. While not a TF, PTEN ranks among the most well-studied proteins in this class. In prostate cancer, *Pten* heterozygous loss promotes proliferation; *Pten* homozygous loss leads to greater proliferation, but only after *Trp53* inactivation to avoid cellular senescence (13, 14). Comparably, in hematopoietic malignancies, inactivation of both copies of *PTEN* results in hematopoietic stem cell depletion, and additional gene mutations are necessary to avoid this loss (14, 15). Even a small 20% reduction in PTEN protein levels can promote tumor development (16). Likewise, subtle, subdiploid reduction in the expression of the TF PU.1 (*SPI1*) is sufficient to promote a preleukemic state (17). Furthermore, in prostate cancer, mutation of *PTEN* is less common than copy number alteration of the locus containing *PTEN*, 10q23. This latter phenomenon may be related to the fact that in addition to *PTEN*, there are five other tumor suppressive genes at this locus that contribute to the phenotype in prostate cancer (18).

The above examples allow us to underscore some principles of HITFs, which we discuss further (**Figure 1**):
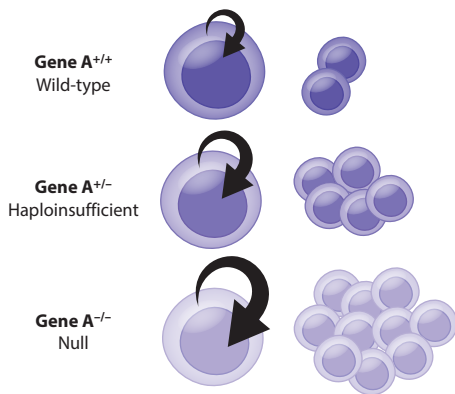
- Principle 1: One is enough, but sometimes two is better—HITFs may exhibit haploinsufficiency in that loss of one allele produces a phenotype, but in some instances loss of the second allele produces a greater or distinct effect.
- Corollary to principle 1: Myeloid neoplasms require a minimal myeloid cell identity—TFs involved in the terminal differentiation of myeloid cells (e.g., RUNX1, CEBPα, PU.1) are rarely mutated to zero remaining activity. This is not true of TFs that are not important in myeloid cell identity (e.g., TP53).
- Principle 2: There are more than three doses—phenotypes associated with the dose of a TF can occur on a continuum of TF activity and are not limited to the Mendelian diploid, haploid, and null levels.
- Principle 3: The monoallelic loss of multiple genes is cumulative—the concurrent losses of genes neighboring a HITF in the setting of aneuploidies and focal deletions can modulate the phenotype.
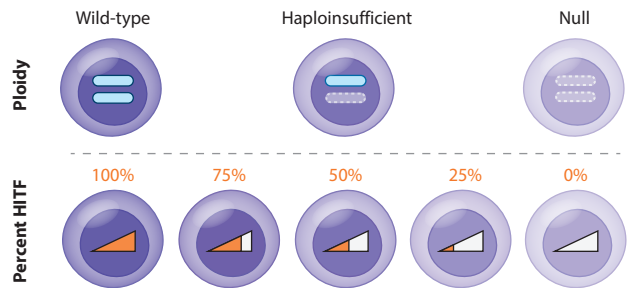
3. DOSE IN HEMATOPOIESIS

The classical, branching tree–based model of hematopoiesis presupposes discrete steps at which developing cells make unforeseen and binary fate choices. While a helpful tool in conceptualizing the hierarchy of development, this model fails to capture the heterogeneous and continuous nature of hematopoiesis (19). Prospective fractioning of hematopoietic stem cells (HSCs) on the basis of cell surface or cytoplasmic protein expression reveals the cell-intrinsic heterogeneity in key HSC functions, namely, cell cycle participation, lineage bias, and self-renewal capacity (20). HSC heterogeneity is further confirmed by retrospective lineage tracing models that demonstrate functional and transcriptional differences present in HSCs (21, 22). In other words, lineage biases are already present at the apex of hematopoiesis, are continuously accrued, and sometimes entail more than one route to arrive at a terminal differentiated cell (23, 24). TFs and coregulators are the likely origins of this cell-intrinsic HSC heterogeneity, as differentiation results from the proper expression and timing of TFs (25).

Imagining the three properties of HSC heterogeneity—self-renewal capacity, cell cycle participation, and lineage bias—as continuous spectra upon which HSCs can reside rather than as discrete phenotypes that cells either possess or do not possess is more consistent with the continuous model of hematopoiesis and the dose-dependent nature of TFs (**Figure 2**). Loss of one copy
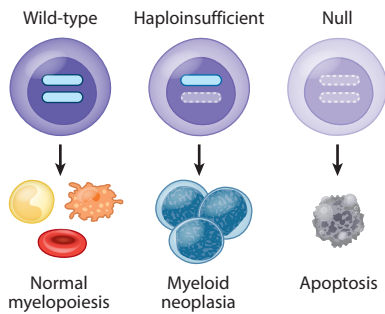
a HITF Principle 1:
One is enough, but sometimes two is better

Gene A$^{+/+}$
Wild-type

Gene A$^{+/-}$
Haploinsufficient

Gene A$^{-/-}$
Null

c HITF Principle 2:
There are more than three doses

Wild-type   Haploinsufficient   Null

Ploidy

100%   75%   50%   25%   0%

Percent HITF

b Corollary to HITF Principle 1:
Myeloid neoplasms require a minimal
myeloid cell identity

Wild-type   Haploinsufficient   Null

Normal   Myeloid   Apoptosis
myelopoiesis   neoplasia

d HITF Principle 3:
The monoallelic loss of multiple genes is cumulative

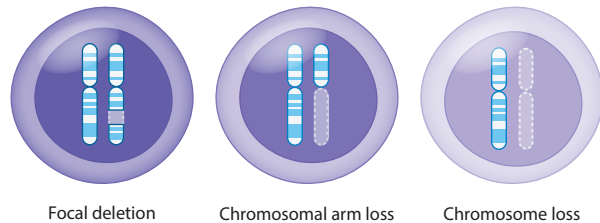Focal deletion   Chromosomal arm loss   Chromosome loss

Figure 1

Principles of HITFs. (*a*). Some genes are haploinsufficient in that monoallelic loss in a diploid organism produces a phenotype. However, this does not preclude an increased effect or distinct phenotype upon a further reduction in activity or loss of the second allele. (*b*) Differentiation into the full panoply of myeloid cells is possible for progenitors with a fully intact myeloid transcriptional network (*left*). A block in myeloid differentiation is imposed with loss of a HITF involved in the differentiation of myeloid cells, and leukemogenesis can proceed with the acquisition of additional genetic aberrations (*center*). However, in the absence of myeloid cell identity, for example, through the total ablation of CEBPα activity, myeloid leukemogenesis cannot proceed (*right*). (*c*) Dose has traditionally been conceptualized in the number of intact alleles of a gene. However, further evidence has given rise to a model of TF activity that extends beyond diploid, haploid, and null. Specifically, preleukemic states can arise in cells with slight, subdiploid alterations in dose of a HITF, and subhaploid but not null doses of a HITF efficiently drive tumorigenesis. (*d*) HITFs are only part of the story when it comes to the phenotype of deletion events that result in the loss of multiple genes. The ultimate cellular phenotypes of deletion events depend on the other codeleted genes. Abbreviations: HITF, haploinsufficient transcription factor; TF, transcription factor.

of a TF can reposition cells along these axes and potentially endow a cell with increased capacity for self-renewal and/or a block in lineage-specific differentiation. For example, monoallelic loss or mutation of the nonclustered, homeobox domain-containing TF CUX1 is found in approximately 10–15% of myeloid neoplasms (**Table 1**) (26, 27). Loss of CUX1 in mouse models promotes an expansion of hematopoietic stem and progenitor cells (HSPCs), progressive anemia, and an expansion of mature myeloid cells, leading to a lethal myelodysplastic syndrome (MDS) (28, 29). In other words, the change in CUX1 dose influences HSPC decisions and moves the HSPCs along the axes. Similar changes can be conceptualized for the other TFs discussed in this review.
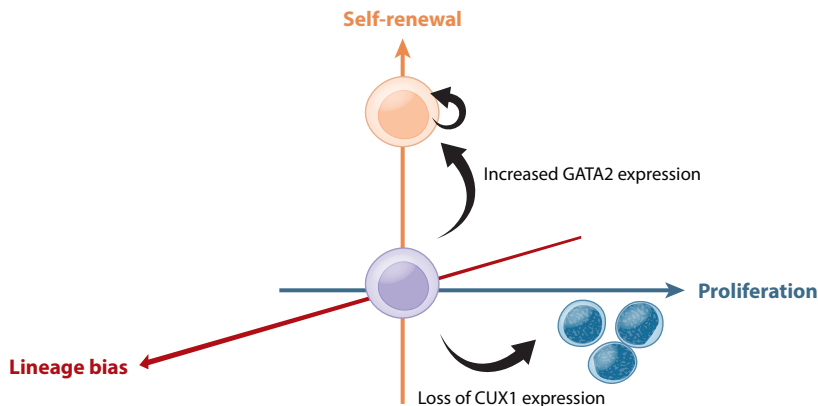
Figure 2

Stem cell properties are continuous characteristics. Self-renewal, proliferation, and lineage bias are properties of hematopoietic stem cells that exist as continuous variables. These properties are dictated by the dose of HITFs. Therefore, alteration of the dose of a HITF can have profound effects on these properties. For example, GATA2 overexpression, which is found in primitive AMLs, results in excess self-renewal. In addition, loss of CUX1 results in increased proliferation and a myeloid bias at the expense of erythroid development. Abbreviations: AML, acute myeloid leukemia; HITF, haploinsufficient transcription factor.

4. MECHANISMS OF TRANSCRIPTION FACTOR HAPLOINSUFFICIENCY

4.1. Transcription Factor Dynamics

TFs actualize the final cell state by reciprocally reading and influencing the enhancer code to promote the expression of effector genes. Transcription is a dynamic and noisy process that depends on the genomic colocalization of many factors. Thus, fundamentally, transcription is a concentration-dependent process. The noise of transcription refers to the phenomenon where, rather than stable, consistent RNA polymerase II (Pol II) activity at a given gene, Pol II transcribes the gene in short, probabilistic bursts, which are influenced by enhancers and TFs (30). *PU.1*, *GATA1*, and *GATA2* mRNA all exhibit noisy transcription with all stem cells exhibiting some expression of these genes, although not necessarily at the same time. Perhaps counterintuitively, these dynamic properties may increase the stability of stemness in the HSC pool as a whole by maintaining HSCs that are sampling every transcriptional state (31).

Transcriptional noise impacts HITFs on two ends: the transcription of the TF itself and transcription of the downstream target genes. TFs generally have low expression levels at baseline (32). Further loss of one TF allele portends particularly noisy expression, and the amount of the TF may even intermittently approach the null state as a result (33). While transcription is inherently noisy, monoallelic loss of a HITF amplifies this noise, resulting in an inconsistent transcriptome and increased intercellular heterogeneity (34). This variation is the precondition for selection, allowing a rare cell transcriptional state to be reinforced at the chromatin level, stabilizing an increase in fitness of a preleukemic cell (35, 36).

In summary, the loss of a HITF has two major ramifications for leukemogenesis (**Figure 3**): First, it provides the requisite myeloid differentiation block. Second, it increases the intercell heterogeneity by increasing the number of sampled transcriptomes, giving the cell an advantage in adapting to the environment.

In addition to increasing transcriptional noise, suboptimal TF concentrations impede the efficiency of TFs binding to cognate DNA sequences. TF dynamics are determined by search pattern,

Myelodysplastic syndrome (MDS): hematologic neoplasms with clonal hematopoiesis, cytopenia(s), and abnormal cellular morphology that can be defined by morphology or recurrent genetic abnormalities

Enhancer: a *cis*-regulatory genomic element that influences the transcription of a target gene

Transcriptional noise: variations in transcription of a given gene between cells of the same type arising from the bursting and probabilistic nature of transcription initiation

Table 1 Frequency of HITF genetic aberrations and genetic aberrations affecting the dose of HITFs across the spectrum of myeloid diseases

Gene	Genetic aberration	CH	CCUS	CMML	MPN	MDS	AML	t-MN
CEBPA	Mutation	0%	0%	1%	0%	2%	9%	4%
CUX1	Mutation	0.03–0.4%	3.4%	10%	0.2–3%	2–6%	1%	2%
	−7/del(7q)	0.0002%	NR	1–10%	0%	2–16%	9–14%	33–50%
EGR1	Mutation	NR	NR	0%	NR	NR	0.5%	NR
	del(5q)	0.0003%	NR	1.5%	0.5%	4–15%	7–16%	49%
ETV6	Mutation	0.1%	0%	0%	1.6%	3–6%	1%	1%
	−12/del(12p)	0.0001%	NR	1%	0.2%	3–5%	2–7%	12%
GATA1	Mutation	0–0.1%	0%	0%	0%	<1%	0.2%	0%
GATA2	Mutation	0–0.1%	1.1%	0%	0.2–2.6%	2–5%	2–3.5%	1%
	inv(3)	NR	NR	NR	NR	Rare	1–1.5%	2%
MYBL2	Mutation	NR	NR	0%	0.7%	NR	1–5%	NR
	−20/del(20q)	0.001%	NR	1%	0%	4–5%	2–6%	9%
RUNX1	Mutation	0–0.2%	3.4%	13.2%	0.5–2%	9–14%	6–12%	10%
	t(8;21)	NR	NR	ED	ED	ED	1–4%	1%
	t(3;21)	NR	NR	NR	NR	NR	0%	3.6%
	inv(16)	NR	NR	ED	ED	ED	3–6%	1–2%
	+21	0.00025%	NR	1%	NR	2–4%	2–5%	11%
TP53	Mutation	2.6–4.4%	1.1%	1%	2–8%	6–13%	6–9%	25–37%
	−17/del(17p)	0.0002%	NR	1%	2%	7%	7%	13%
PML-RARA	t(15;17)	NR	NR	ED	ED	ED	3–9%	2%
Selected mutations that alter the dose of HITFs								
MLL1	t(11q23;x)	NR	NR	NR	NR	NR	3%	3.3%
NPM1	Mutation	0–0.1%	0%	2.6%	0.7%	1.5–2%	22–27%	15%
FLT3	Mutation	0–0.1%	0%	2.6%	0.5%	1.5–5%	28–33%	20%
	Reference(s)	78, 177–179	180	92, 177, 181–183	183–185	183, 186–188	92, 122, 130, 189–192	94, 122, 145, 193

Abbreviations: AML, acute myeloid leukemia; CCUS, clonal cytopenia of undetermined significance; CH, clonal hematopoiesis; CMML, chronic myelomonocytic leukemia; ED, excluded on the basis of diagnostic criteria; HITF, haploinsufficient transcription factor; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NR, not reported; t-MN, therapy-related myeloid neoplasm.

search time, and on/off rates (25). The search pattern of a TF refers to how the protein moves about the nucleus to find target sequences. MYC, for instance, explores the entire nucleus, while other TFs, such as CTCF, linger in smaller areas containing a high concentration of binding sites (38, 39). TF search time refers to the interval between DNA binding events and can be influenced by mitigating factors such as the chromatin milieu (40). TFs spend the most time unbound and in search mode (25). Per the law of mass action, a reduction in TF molecules will lower the overall likelihood that the DNA targets are occupied by the HITF. While this conclusion is intuitive, the downstream consequences can be complex. For instance, TFs regularly interact with other TFs and/or cooperatively bind DNA. SOX2 is one such example and guides OCT4 to cobind DNA. Decreased SOX2 concentration not only curtails SOX2 DNA targeting but also increases the search time of OCT4 (41). Numerous TFs physically interact with one another and bind DNA in concert, suggesting that they could have linked search patterns as well. Prolonged searching for DNA binding sites could be further exacerbated in situations where the HITF is a pioneer
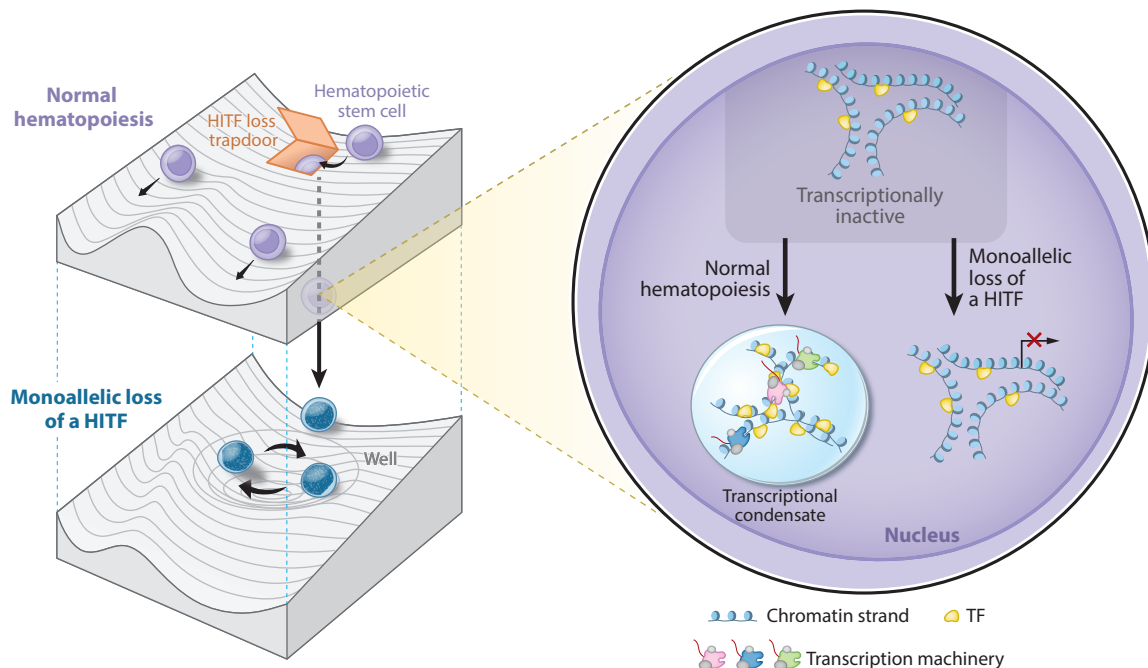
Figure 3

Loss of a HITF results in a myeloid differentiation block and greater transcriptional plasticity. HSCs can develop normally by following one of the differentiation paths out of the bone marrow. Or, in a variation on Waddington's landscape (37), HSCs can undergo monoallelic loss of a HITF, represented as falling through the trapdoor. The loss of a HITF has profound effects on the nuclear environment. Normally, TFs bridge DNA sequences at enhancers with the general transcription machinery to hold all of these molecules in close proximity to transcribe a gene. However, with decreased TF molecules present in the nucleus, clustering of all the requisite DNA sequences and transcriptional machinery happens less frequently. It is difficult to achieve a sufficiently high local concentration of a TF to move enhancers and genes into an environment permissive for transcription, which can result in genes failing to be relocated and thus transcribed. Consequently, increased transcriptional plasticity ensues as cells fail to maintain a consistent epigenome and transcriptome. Furthermore, such cells have an inability to properly differentiate to myeloid cells due to the dysregulated enhancer landscape. These two ramifications of HITF loss—increased transcriptional plasticity and a myeloid differentiation block—are depicted as the cell rolling around in the well, sampling many different transcriptomes, and being unable to escape down the path of normal myeloid differentiation. Abbreviations: HITF, haploinsufficient transcription factor; HSC, hematopoietic stem cell; TF, transcription factor.

factor, wherein many TFs depend on the HITF for access to DNA. These scenarios highlight the wide-ranging detriments associated with inappropriate concentrations of a HITF.

Even in normal conditions, cells temporally manipulate the nuclear concentration of TFs to bring about distinct cellular fates. This phenomenon is exemplified by nuclear hormone receptors, which shuttle into the nucleus upon ligand binding, but also extends to other classes of TFs (42). For instance, the effects of the dosage-sensitive TF TP53 are conveyed by nuclear localization. Pulses of TP53 into the nucleus promotes the repair of DNA damage, whereas sustained TP53 nuclear localization causes senescence (43). In this example, the extent of DNA damage is communicated through the nuclear concentration of TP53, producing different outcomes. The developmental TF HES1 also exhibits distinct nuclear oscillation patterns and concentrations, producing different outcomes in neural progenitors (44). Nuclear factor kappa B (NFκB) signaling patterns in murine HSPCs similarly result in differential gene expression of developmental or apoptosis genes in response to inflammation (45). Given the finely tuned patterns necessary for these TFs to convey unique cell state information, it is unlikely that only one allele of a TF could achieve these

same nuanced dynamics. These examples indicate not only the importance of TF concentration but also the ability of dose dynamics to convey different responses to environmental stimuli.

4.2. Clustering the Characters

Context-dependent gene expression is largely mediated by enhancers (46). To influence transcription, an enhancer must have sustained interaction with a target promoter, and TFs bestow DNA sequence specificity on this interaction (41). One enhancer can regulate multiple genes, and multiple genes can be coordinately regulated in turn, a process bridged by TFs (41, 47, 48). These actors can be found in physical proximity to each other and have been described as transcriptional hubs or factories (48–51). More recently, liquid-liquid phase separation has been considered an etiology of nuclear subcompartmentalization (52). These biophysical structures may explain the observed clustering of the requisite transcriptional regulators and *cis*-regulatory elements that regulate Pol II activity. Much more work is warranted to understand the role of phase separation in transcriptional regulation. Nonetheless, in both the hub and condensate models, TFs migrate into subcompartments of the nucleus, increasing local TF concentration (49, 51, 53, 54).

KLF1 and EBF1 provide two examples of how TFs can be important for the relocation of target genes. In murine erythroid progenitors, KLF1 forms dynamic transcription factories. KLF1 shepherds together erythroid genes from across the genome, including hemoglobin genes, to coordinately drive transcription promoting erythropoiesis (49). Reiterating the importance of the proper nuclear localization of TFs and target genes, monoallelic loss of the B cell HITF EBF1 results in less efficient relocation of a target transgene out of heterochromatin, leading to decreased transgenic expression. In this scenario, there is insufficient EBF1 to pull the transgene into a nuclear environment more conducive for transcription (55). This finding supports a model of transcription where the TF must attain a certain local concentration at a given locus, which is associated with moving the gene into a permissive transcriptional environment. This model presupposes the ability to generate high local concentrations of a TF, a feat that is impaired in the setting of haploinsufficiency. The decreased frequency of activation of the transgene underscores the probabilistic nature of transcription and the buffering capacity of two alleles.

The biochemical properties mediating the clustering of *cis*-regulatory elements and the general transcription machinery is an active area of investigation. Consequently, intrinsically disordered regions (IDRs) have received renewed attention for the ability of these protein domains to mediate clustering, condensation, and liquid-liquid phase separation. The overwhelming majority of TFs have IDRs, which lack fixed three-dimensional structure but can still convey interaction specificity (56, 57). Mutations in IDRs are common in cancer and enriched in known drivers of hematopoietic malignancy (58). The transactivation domains of TFs have repeatedly been shown to be IDRs, which form condensates with general transcription machinery, including Pol II, Mediator, and BRD4, leading to high local concentrations of the important players for transcription (51, 59–63). These findings lead to a model wherein the IDRs and DNA binding domains of TFs serve to couple the transcriptional machinery with DNA. As a result, the TFs are held in increased local concentration to facilitate repetitive DNA binding and transcriptional bursting (50, 52, 64, 65). The loss of one copy of a HITF decreases the probability of target gene compartmental sequestration and activation. Overall, sequestration of transcriptional players and processes within the diffusion space of transcription condensates reduces transcriptional noise, as important molecular players are more likely to bump into each other (66).

4.3. Models of Transcription Factor Action at Target Genes

Regardless of the physical relationship between TFs, enhancers, and transcription initiation, some genes are more sensitive than others to diminished TF levels, begging the question of how a TF

Transcription condensates: assemblies of high local concentrations of the machinery required to transcribe a gene (TFs, transcription coregulators, and RNA Pol II) and their subsequent dissolution

segregates in the nucleus under limiting conditions. For example, in murine HSPCs, heterozygosity of *RUNX1* leads to a loss of binding to and expression of the *Cebpε* locus but not the *Cebpα* locus, suggesting an underlying mechanism governing the actions of dose-limited RUNX1 (67). The differential sensitivity of some genes to the loss of a TF has led some investigators to conceptualize TFs as having either analog or digital outputs. RUNX1 in this case is functioning in a digital or binary manner; at a certain threshold of expression, RUNX1 either activates a gene target or does not. The TF ETV2 also exhibits digital effects. ETV2 can promote the development of both endothelial cells and erythroid cells, and this dichotomy is conveyed through different thresholds of binding and activation for genes of each lineage. Specifically, low levels of ETV2 are sufficient to promote expression of endothelial genes, but a much higher threshold must be met to promote erythroid gene expression (68). In contrast, NFκB TFs exhibit analog or gradient-like effects where the expression of target genes is directly proportional to the level of NFκB activation (69). At a limited number of loci tested, PU.1 also appears to act in an analog manner at loci related to proliferation (70). Interestingly, *SOX9*, which is mutated in devastating craniofacial developmental disorders, exhibits both analog and digital activity. Enhancers that exhibit analog activity are more likely to have a SOX9 palindrome motif and are associated with different pathways than digital enhancers, which are more likely to have a weak SOX9 motif and be bound by other TFs (71). This later work represents the most advanced analysis of a dose-dependent TF, accomplished through the use of a degron tag to generate a spectrum of TF doses. As such tools are further developed to manipulate TFs at a single-cell level in an inducible manner, we may find that more TFs resist simplified classification into analog and digital regulators.

4.4. Compensation

In addition to the biochemical sequestration that condensates provide, cells have other mechanisms to safeguard the robustness of the transcriptome. In some cases, however, these mechanisms may be insufficient to compensate for the monoallelic loss of a HITF. The long half-lives of mRNA molecules and posttranscriptional regulators are thought to buffer against the probabilistic nature of transcription. However, TFs are generally less stable on the mRNA and protein levels as compared with the rest of the genome (32). Hence, compensatory mechanisms are less efficacious for TFs.

Some proteins have closely related family members that can minimally compensate for the loss of one of the members. With respect to RUNX1, family members RUNX2 and RUNX3 can partially compensate for the loss of RUNX1 (72). In the setting of CEBPα loss, CEBPβ (*CEBPB*) can compensate in the setting of emergency granulopoiesis induced by inflammation (73). Yet, as a class, HITFs are less likely to have paralogs, contributing to the necessity of two alleles of these genes for proper development (74).

5. HOW TO LOSE A PARTNER IN SEVEN WAYS

Having considered the molecular mechanisms of TF action and the implications for haploinsufficiency and dose dependence, we now focus on the common means by which the abundance of a gene product can be altered and how the mechanism of gene inactivation can have a profound impact on the phenotype manifested (**Figure 4**). For example, the chromatin landscape of RUNX1-translocated acute myeloid leukemia (AML) is counterintuitively more akin to that of double-mutant CEBPα AML (CEBPα^{DM}) than AMLs with RUNX1 point mutations (75). Below, we consider the different types of HITF loss and how the context impacts the phenotype.

5.1. Aneuploidy

Losing one copy of part or all of a chromosome is seemingly the most straightforward method to lose one allele of a TF; however, the implications of losing potentially hundreds of neighboring
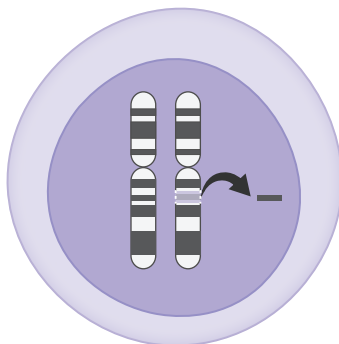
Acute myeloid leukemia (AML): the clonal expansion of malignant HSPCs defined by genetic aberration or extent of differentiation of blasts
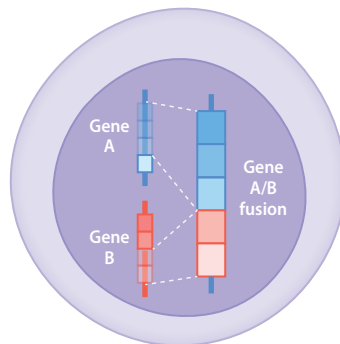
a Aneuploidy
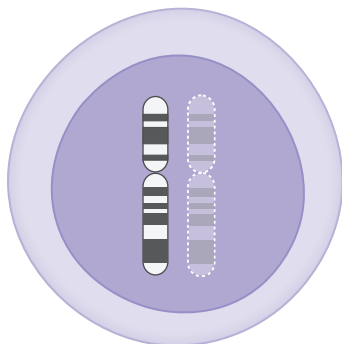
Examples:
−7/del(7q), del(5q), del(20q),
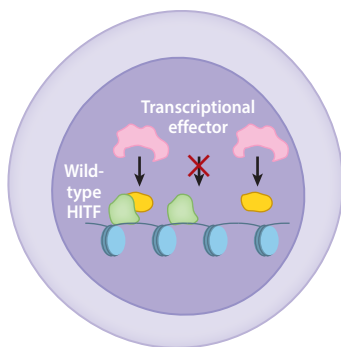del(12p), −17/del(17p)

b Focal deletion

Examples:
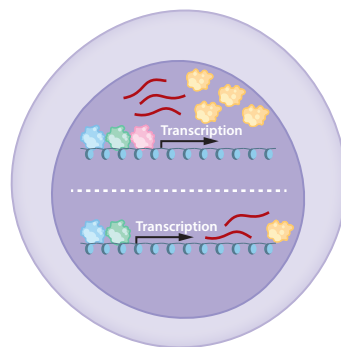7q22, 7q36

c Balanced translocations
and inversions

Gene
A

Gene
A/B
fusion

Gene
B

Examples:
t(15;17), inv(3), t(8;21), t(3;21), inv(16)

d Mutation

Transcriptional
effector

Wild-
type
HITF

Examples:
RUNX1, TP53, GATA1, GATA2

e Mutation of another gene

Transcription

Transcription

Examples:
PU.1, RUNX1

f Change in isoform balance

Isoform A

Isoform B
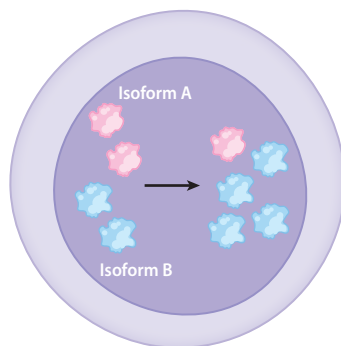
Examples:
CEBPA, RUNX1

g Epigenetic silencing

HIFT

Me Me Me Me Me Me
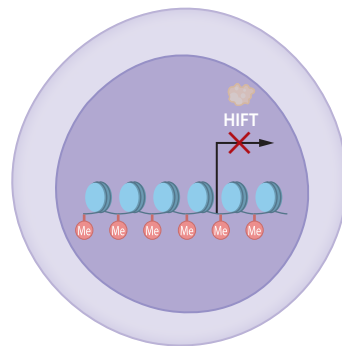
Example:
GATA2

(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Multiple ways to lose a HITF. (*a*) Aneuploid events result in the loss of a HITF in the context of the loss of an entire chromosome or chromosomal arm. (*b*) Focal deletions result in the loss of a HITF without the ensuing cellular stress of aneuploidy, but the phenotype is still the consequence of the loss of multiple codeleted genes. (*c*) Balanced translocations and inversions create a potent fusion oncogene or lead to the overexpression of an oncogene, but the genetic disruption also creates loss-of-function events in the rearranged, residual genes. (*d*) TFs have two basic parts: the domain that binds DNA and the part(s) of the protein that assembles with cofactors, other TFs, and/or general transcription machinery. Mutations in either of these parts can have diverse effects on the mutant protein. The TF can lose the ability to bind DNA and thus wild-type function (*right*). A TFs can also lose the capacity to associate with necessary cofactors and thus forfeit the ability to serve as a bridge between specific DNA sequences and other proteins to regulate transcription (*center*). Dominant negative proteins arise on the basis of how much the mutant protein interferes with the functions of the remaining wild-type allele. (*e*) The complexity of the myeloid transcription network necessitates that dose alterations in one protein can have cascading disturbances on the effective dose of another gene. Thus, mutation of a HITF or other critical myeloid gene can have profound effects on the transcription or stability of other HITFs. (*f*) TFs have multiple isoforms with different functions. One way that the effective dose of a HITF can be disrupted is a change in the balance of these isoforms away from the wild-type ratios. This departure from the normal ratio changes the function of the TF pool given that the isoforms have different capabilities and functions. (*g*) The final way that HITF dose can be affected is the loss of expression of a HITF through aberrant epigenetic silencing through DNA methylation, repressive chromatin methylation, or a loss of accessibility at important sites regulating the expression of the gene. Abbreviations: HITF, haploinsufficient transcription factor; Me, methylation; TF, transcription factor.

genes in tandem as well have not been sufficiently appreciated (76). In most cases, aneuploidy is a detriment to the fitness of a cell—this is evinced in the finding that the more genes on a chromosome, the less likely cancer cells are to tolerate loss or gain of that chromosome (77). It is only under specific circumstances that aneuploidy confers an advantage. And while aneuploidy has been thought to be a late event of advanced-stage neoplasms, aneuploidy can be an early and founding event, as evidenced by aneuploidy in clonal hematopoiesis (78).

In general, any monosomy will have profound effects on the cell, including inducing ribosomal stress and gene expression changes that are not limited to the affected chromosome. The ensuing proteotoxic stress activates a TP53 response that can promote cell death (79). Consequently, aneuploidy is largely incongruous with intact TP53 signaling, likely due to proteotoxic stress, and this may be why AML and MDS with complex karyotypes often also present with *TP53* mutations (80). That said, while there are common features of aneuploidies, there is variability to the cellular response to aneuploidy as well. In one report, cells harboring an identical aneuploid event can diverge with respect to cell cycle alterations, response to extracellular and intracellular signals, and gene expression (81). This aneuploidy-induced transcriptional plasticity likely also fosters increased drug resistance (82). Others have argued that there is extensive posttranscriptional compensation for aneuploidy in cancer. As a result, protein changes are not as dramatic as one might surmise, including for oncogenes and tumor suppressors (83). Nonetheless, an aneuploid event results in not only the loss of a HITF within a greater genetic deletion but also the concomitant cellular stress of aneuploidy.

A second distinction of aneuploidy-induced HITF loss is that several pathogenic genes may be simultaneously deleted en bloc. A contiguous gene syndrome (CGS) can arise in this event, which refers to the phenotypic consequences of the loss of multiple neighboring genes. It is technically challenging to experimentally model CGS; however, there is mounting evidence that multiple genes encoded on a chromosome arm can cooperatively drive cancer when lost (84). In some cases, CGS genes may converge on similar pathways; in others, genes may impact complementary pathways (85). It would be remiss to neglect codeleted genes when considering HITFs impacted by aneuploidy (HITF principle 3). In the remainder of this section, we highlight HITFs within recurrent aneuploid events in myeloid neoplasms. However, we acknowledge that aneuploidies and focal deletions have more complex phenotypes than solely the loss of the HITF alone.

EGR1 encodes an ETS family TF and is frequently deleted in the context of del(5q). Consistent with del(5q) as a recurrent event in myeloid neoplasms arising in patients with a history

Clonal hematopoiesis: the overrepresentation of mature blood progeny derived from at least one mutant hematopoietic stem cell clone and the absence of a detectable hematological abnormality

Contiguous gene syndrome (CGS): a disorder arising from the loss of multiple neighboring genes in a single genetic event resulting in a phenotype that represents the combinatorial haploinsufficiency of those genes

of cytotoxic therapy [therapy-related myeloid neoplasms (t-MNs)], monoallelic loss of *EGR1* is sufficient for myeloid neoplasia upon treatment with an alkylating agent (86). The loss of *EGR1* conveys a fitness advantage by blunting the DNA damage and inflammation response in HSPCs (87). Highlighting principle 3, EGR1 cooperates with another 5q resident, *APC*, encoding a Wnt signaling component, as well as *Trp53* to promote de novo AML. In contrast, heterozygosity for only one or two of the three genes does not result in AML (88). This example highlights the aggregate phenotype that can arise due to cumulative haploinsufficiency and emphasizes the need to consider the impact of genes deleted concomitantly with the HITF.

CUX1, a homeodomain-containing TF, has conserved dose sensitivity from *Drosophila melanogaster* to humans. In flies, the homologue of CUX1, Cut, has dose-dependent roles in the development of at least two organ systems (89, 90). Interestingly, up to four different doses of Cut are associated with different developmental fate decisions, suggesting that manipulation of the level of this TF is one way that developmental decisions are made. Different fates associated with different doses of CUX1 may be realized by the analog nature of CUX1 (91). The graded effects of CUX1 dose are true at the level of not only transcription but also the elicited phenotype. In mice, knockdown of CUX1 to an intermediate level results in a mild form of MDS with anemia, while further knockdown results in a more severe anemia, an expansion of mature myeloid cells, and a lethal form of MDS/myeloproliferative neoplasm (MPN) (29). Certain features unify CUX1 and its orthologues across model systems and human disease: Knockdown of CUX1 results in changes in proliferation and impaired differentiation (27, 29). In humans, *CUX1* is deleted in the context of −7/del(7q) without silencing or mutation of the remaining allele (26, 27, 92). In de novo myeloid malignancies, *CUX1* mutations or −7/del(7q) are more associated with MDS. In fact, in some cases of pediatric MDS, −7/del(7q) appears sufficient for the disease, as it is the sole somatic genetic change identified (93). Also, −7/del(7q) is strongly associated with t-MNs (94). One explanation for this association is that CUX1 has an early role in the DNA damage response; hence, CUX1 deficiency aberrantly allows continued HSPC proliferation in the setting of DNA damage stemming from cytotoxic therapy (95). The resultant clonal advantage of CUX1-deficient cells ultimately leads to the development of an overt myeloid neoplasm. This mechanistic work highlights the role of the HITF levels in normal differentiation and how deviant levels promote transformation.

Del(20q) results in the loss of *MYBL2* (bMyb), a TF that regulates the differentiation and self-renewal of myeloid progenitors (96). Homozygous deletion of *MYBL2* is embryonic lethal; however, haploinsufficiency drives a range of myeloid disorders, including MDS and MPN (97). Downregulation of *MYBL2* is sufficient for murine HSPC expansion, which is consistent with *MYBL2* downregulation as a common feature of MDS, regardless of 20q status (98). This finding indicates that del(20q) is not the only route to downregulate *MYBL2*.

Del(12p) is a small interstitial deletion found in MDS with a complex karyotype. Due to its small size, del(12p) is underreported in standard cytogenetic analyses (99). The ETS-family TF ETV6 is lost in del(12p) events, and the remaining allele is generally not methylated or mutated, cementing this TF as a HITF (99, 100). ETV6 is a homodimeric transcriptional repressor required for the establishment of hematopoiesis and survival of adult HSCs (101). Like many of the other HITFs discussed here, including *RUNX1*, *CEBPα*, and *GATA2*, *ETV6* also harbors germline mutations (102). Similar to individuals carrying a germline *RUNX1* mutation, patients with inherited *ETV6* mutations have a high likelihood of developing thrombocytopenia due to defects in megakaryocyte and platelet maturation, underscoring the necessity of both alleles of these two genes for proper platelet generation (103).

Unlike the genes we have considered thus far, *TP53* frequently harbors biallelic alterations. *TP53* alterations include mutations, loss of heterozygosity, and deletions in the form of

−17/del(17p). As *TP53* status is usually assessed amid full-blown malignancy, when biallelic alterations are common, *TP53* can appear to abide by the two-hit hypothesis (104). Even so, there is ample evidence from mouse and human studies that monoallelic loss of *TP53* is sufficient to promote leukemogenesis. *Trp53* heterozygosity is conducive to murine HSPC clonal outgrowth after DNA damage (105). Likewise, heterozygosity for *Trp53* is permissive for tumor formation in mice, and the remaining allele can remain intact and functional (106). In MDS, while many patients have monoallelic or biallelic *TP53* mutations, a small subset of patients present with loss of 17p without mutation or loss of heterozygosity of the remaining allele (104). Notably, like other aneuploid events, del(17p) deletes additional tumor suppressor genes that independently drive tumorigenesis in mice (84). Hence, our understanding of del(17p) pathogenesis remains incomplete. Notwithstanding, these studies indicate that TP53 produces haploinsufficient phenotypes that may become more pronounced with biallelic loss (HITF principle 1).

5.2. Focal Deletions

In contrast to aneuploidy, focal deletions are smaller than a chromosome arm (77). Focal deletions can affect HITFs while potentially avoiding some of the fitness defects induced by aneuploidy. In fact, there is selection to delete tumor suppressor genes while preserving essential genes and oncogenes in focal deletions (107). As a result, focal copy number alterations are more potent in driving proliferation signatures as compared with chromosome arm level changes (108). That said, focal copy number alterations still correlate with mutations in DNA damage response genes. This latter finding implicates the DNA damage response pathway as a gatekeeper for genomic instability in general, as remarked upon above with *TP53* mutations and aneuploidy (108).

Also analogous to aneuploidy, HITF principle 3 remains true for focal copy number changes. As an example, we return to del(7q). In addition to monosomy 7 and del(7q), smaller cryptic deletions can occur that are below the sensitivity of standard cytogenetics analysis. Higher-resolution approaches have revealed minimally deleted regions, including one spanning *CUX1* on 7q22 (27, 109). While *CUX1* loss is sufficient to cause myeloid disease, deletion of a 7q22 syntenic region in mice that does not include *CUX1* also disrupted hematopoiesis (29, 110). These data implicate more than one haploinsufficient 7q22 gene. Furthermore, these findings of a CGS in a focal deletion extend to other copy number variations as well (111).

5.3. Balanced Translocations

Balanced translocations typically garner attention for the ensuing fusion product or overexpressed oncogene. For instance, t(15;17)(q22;q12) produces the PML-RARA fusion protein, pathognomonic for acute promyelocytic leukemia (APL). Less appreciated is the fact that such translocations inherently generate loss of function in the rearranged genes, in this case, PML and the TF RARA. Translocations can thereby result in three hits for the price of one. While the fusion product alone initiates leukemogenesis, haploinsufficiency of PML and RARA also further promotes disease (112, 113).

Similarly, inv(3)(q21q26.2) or t(3;3)(q21;q26.2) repositions the *GATA2* distal enhancer away from *GATA2* to instead drive oncogenic overexpression of the EVI1 TF (114). Inherently, this inversion or translocation also attenuates *GATA2* expression. Indeed, GATA2 heterozygosity accelerates EVI1-mediated oncogenesis in mice (115). A minority (15%) of patients also have mutations of the nonrearranged *GATA2* allele (HITF principle 1) (116). Thus, like t(15;17), inv(3) simultaneously produces both gain-of-function oncogenic events and loss-of-function events in HITFs.

RUNX1 is another HITF involved in balanced translocations. RUNX1 is a pleiotropic TF required for the initiation of definitive hematopoiesis and multilineage differentiation in adult hematopoiesis. However, RUNX1 does not work alone; it requires the cofactor CBFβ (*CBFB*) to

Essential gene: a gene for which a loss-of-function event results in severely impaired viability or fitness for a cell or organism

Gain-of-function: a mutation in which the mutant allele possesses a de novo function absent from the wild-type allele

achieve proper regulation of target genes (117). There are three ways that this critical interaction can be interrupted through translocation to promote the development of myeloid malignancy: Two are translocations involving *RUNX1* and one is a translocation involving *CBFB*.

The first translocation is the t(8;21)(q22:q22.1) translocation, which fuses the RUNX1 DNA-binding Runt homology domain (RHD) to the Nervy-homology region (NHR) domains of ETO (RUNX1T1). The NHR recruits the repressive nuclear receptor corepressor (NCoR) complex (118). Consequently, this translocation results in a neomorphic RUNX1 fusion protein that re-presses myeloid differentiation genes it normally activates, such as *PU.1*, *GATA1*, and *CEBPα* (119–121). The second translocation is t(3;21), which is most commonly found in t-MNs; t(3;21) fuses the RHD of RUNX1 to the TF EVI1. RUNX1-EVI1 also results in the loss of *CEBPA* expression, and thus the promyeloid differentiation program, but uniquely upregulates *GATA2*, producing a more primitive AML (122, 123). Both translocations create a potent oncoprotein and simultaneously disrupt the involved *RUNX1* allele. The derivative RUNX1 cannot interact with CBFβ to drive proper gene expression and is effectively dysfunctional. The remaining wild-type RUNX1 is in competition with the oncogenic RUNX1 fusion for DNA binding sites, resulting in a finely tuned transcriptional network optimized for cellular fitness (124). This is made apparent upon loss of the wild-type *RUNX1* allele, as knockdown of RUNX1 in cells expressing RUNX1-ETO causes apoptotic cell death due to the overactivity of RUNX1-ETO (125). The remaining wild-type RUNX1 activity is seemingly subhaploid but absolutely critical, highlighting the dose-dependent actions of RUNX1 (HITF principle 2).

The final translocation disrupting RUNX1 dose is inv(16)(p13q22), which rearranges *CBFB*, the gene encoding the obligate RUNX family TF cofactor, CBFβ. Inv(16) fuses *CBFB* to *MYH11* (SMMHC); the resultant CBFβ-SMMHC oncoprotein retains the ability to bind RUNX1. CBFβ-SMMHC actually has greater affinity for RUNX1 than CBFβ alone and co-opts RUNX1 to drive the leukemogenic program (126). Wild-type RUNX1 activity is diminished but not abolished as a result. In fact, some residual RUNX1 activity is essential for the survival of this AML (125, 127, 128). Further suggesting reduced RUNX1 dose as important in this disease, monoallelic loss of *Runx1* in mice phenocopies inv(16) AML with respect to granulocyte maturation failure (127). In addition to disrupting RUNX1 function, CBFβ-SMMHC reduces TP53 dose by indirectly mediating the inappropriate deacetylation and inactivation of TP53 (129). Thus, the mechanism of pathogenesis of inv(16) AML is the result of the oncogenic fusion protein compounded by the interference with two dose-dependent TFs, TP53, and RUNX1.

5.4. Mutations

Mutations are not created equal and can have widely varying effects on protein function, so we must consider the details of individual mutations to understand how they affect the dose of a given TF.

RUNX1 is among the most frequently mutated TFs in AML and MDS (130). Patients with familial platelet disorder have inherited loss-of-function mutations in *RUNX1*, which results in cell-intrinsic impairment of megakaryocyte differentiation and an increased risk of myeloid malignancy (131). Expectedly, the loss of one copy of *RUNX1* in induced pluripotent stem cells (iPSCs) results in compromised megakaryocyte differentiation (132). *RUNX1* mutations exist on a functional spectrum. N-terminal mutations result in pure loss of function, as they result in truncations or mutations that disrupt the ability to bind DNA or CBFβ. Mutations in the DNA binding domain (RHD) are weakly dominant negative. Finally, mutations in the C-terminal transactivation domain are strongly dominant negative (80% reduction in RUNX1 activity) (133). The precise mechanism of strong dominant negative mutations is unclear and could be multifactorial.

Dominant negative: a mutation in which the mutant allele negatively affects the wild-type allele, producing a consequence greater than a loss-of-function variant in one allele alone

Underscoring the exquisite dose sensitivity of RUNX1, loss of one copy of *RUNX1* or a weak dominant negative mutation (R174Q) are both associated with thrombocytopenia and decreased megakaryocyte differentiation in iPSC culture. However, only the strong dominant negative mutation results in an expansion of myeloid progenitors and increased genomic instability (134). Further emphasizing the dose-dependent nature of RUNX1, myeloid malignancies with lower RUNX1 activity have a higher likelihood of secondary leukemic progression (135). The spectrum of RUNX1 activity and associated phenotypes capture the essence of HITF principle 2.

GATA1 is an X-linked gene, so while it cannot be considered classically haploinsufficient, it still exhibits dose-dependent effects. This characteristic is featured in the GATA1^{knockdown} mouse, which displays impaired erythropoiesis and megakaryopoiesis (136). Total GATA1 ablation causes erythroid progenitor apoptosis, while a partial decrease promotes proliferation and a leukemia-like disease, underscoring the dose-dependent nature of GATA1 (137). A second example of this is apparent in Down syndrome. Of infants with Down syndrome, characterized by trisomy 21, 10% develop a self-limiting leukemia-like disease termed transient abnormal myelopoiesis (TAM). Twenty percent of TAM cases will evolve to acute megakaryoblastic leukemia (AMKL). Virtually every case of TAM and AMKL has a mutation in *GATA1* (138). *GATA1* mutations in the context of trisomy 21 are sufficient for TAM but not for AMKL, in which other mutations are acquired. Generally, the mutations in *GATA1* are 5′ nonsense mutations, allowing protein synthesis to restart at a 3′ start codon to create a shorter isoform, GATA1s (139). The GATA1s isoform lacks part or all of the N-terminal transactivation domain, reducing the binding of an important cofactor, FOG1, ultimately leading to a TF with less transactivation capability. In this way, incomplete loss GATA1 activity is pathogenic in TAM and AMKL.

A second GATA family TF member, GATA2, is also a HITF. Loss of one copy of murine *Gata2* results in reduced HSC numbers, increased HSC proliferation, and impaired self-renewal upon transplant (140). Conversely, overexpression of GATA2 results in a differentiation block, suggesting that GATA2 is also triplosensitive (141). The vast majority of mutations in *GATA2* are predicted to generate a loss-of-function allele, via either nonsense or missense mutations in one of the two zinc finger domains (142). Mutations in the first GATA2 zinc finger domain create a loss-of-function allele due to the inability of the mutant protein to bind DNA. These mutations are associated with CEBPα^{DM} in myeloid neoplasms (143). In a mouse model of CEBPα^{DM} and a first zinc finger domain mutation of *Gata2*, the *Gata2* mutation causes decreased accessibility of myeloid TF binding sites, while both TFs increase erythroid TF expression (144). This mechanistic study provides an explanation for the bilineage leukemia that arises from synergistic and concurrent mutations in *CEBPA* and *GATA2*.

We return to consider how TP53 is different than the other TFs discussed in this review. *TP53* mutations are enriched in older patients, t-MNs, and complex karyotype malignancies (145). In myeloid neoplasms, loss-of-function, dominant negative, and gain-of-function *TP53* mutations have been described (105, 146, 147). All of these mutations result in differing degrees of TP53 impairment. There is an inverse relationship between TP53 activity and the ensuing fitness advantage in malignancies. Cells with the least amount of TP53 activity have the greatest fitness advantage, and cells can accordingly gain a further advantage by removing residual TP53 activity. For instance, of MDS cases with *TP53* aberrations, one-third are monoallelic and two-thirds are biallelic (104). Monoallelic *TP53* mutations do not connote the poor prognosis that biallelic mutations carry. In some patients with monoallelic *TP53* mutations, MDS transforms to secondary AML with concomitant additional *TP53* loss. With regards to the clonal structure, patients with monoallelic *TP53* mutations have greater clonal diversity than patients with biallelic *TP53* mutations, and biallelic *TP53* mutations are always in the dominant clone, further indicating the relative advantage of leukemic cells with biallelic *TP53* mutations (HITF principle 1). These results

buttress the notion that monoallelic *TP53* loss is sufficient to drive MDS but is not synonymous with biallelic *TP53* disease.

5.5. Mutation of Another Gene

While rarely mutated itself, the ETS family TF PU.1 is often a concomitant casualty of other genetic aberrations. The centrality of PU.1 in the myeloid transcriptional network means that many common genetic aberrations in AML affect PU.1 dose. Functionally, PU.1 is required for HSCs to generate myeloid progenitors and for myeloid differentiation beyond the granulocyte monocyte progenitor stage (148). In addition to orchestrating differentiation, PU.1 is a regulator of HSC proliferation and protein synthesis. Consequently, loss of PU.1 increases HSC cycling and decreases self-renewal (70). The mouse models of PU.1 illustrate the dose-dependent aspects of this TF. Heterozygous PU.1-deficient mice have various hematopoietic defects but do not spontaneously develop AML (149). However, an 80% reduction in PU.1 by homozygous deletion of an enhancer upstream of *PU.1* results in a transplantable AML (150). Interestingly, heterozygous deletion of this enhancer produces a 35% reduction in PU.1 and a preleukemic state, which progresses to an overt AML on a DNA-repair-deficient background (17). These mouse models demonstrate that varying PU.1 doses from slight to more severe reductions are associated with unique leukemic and preleukemic states (HITF principle 2). In fact, low levels of PU.1 activity are required to form competent leukemic stem cells, as mutation of the RUNX1 binding sites in a *Pu.1* enhancer results in a severe decrease in leukemic stem cell activity and delayed onset of RUNX1-ETO-driven leukemia (72).

Many paths to myeloid neoplasms pass through PU.1, given its requirement in myeloid differentiation and cell cycle control. RUNX1 normally regulates PU.1 expression in a dynamic and lineage-specific manner; thus, alteration of RUNX1 dose has repercussions for PU.1 levels (151). RUNX1 fusion proteins, for instance, not only disrupt the normal function of RUNX1, as discussed above, but also directly reduce PU.1 expression. There are two active ways that RUNX1 fusion proteins reduce PU.1 dose: exploitation of an antisense promoter normally used to downregulate PU.1 during T cell development, and inactivation of a long noncoding RNA that normally facilitates PU.1 expression (152, 153). In addition, some N-terminal *RUNX1* mutations abrogate RUNX1 interaction with and recruitment of the activating H3K4 methyltransferase MLL (*KMT2A*) to the PU.1 promoter (154). Moving beyond RUNX1, PU.1 levels are low in APL and increase after ATRA-induced differentiation; in fact, overexpression of PU.1 is sufficient to promote differentiation in this model (155). Mutations in *CEBPA* result in the downregulation of PU.1, and the FLT3 internal tandem duplication mutation results in the downregulation of PU.1 as well as the downregulation of CEBPα (72, 156–158). Although different groups have found conflicting results, mutation of the nucleolar protein NPM1 results in a strong nuclear export signal and can drag PU.1 along with it into the cytoplasm (159, 160). Finally, a reduction in PU.1 expression accelerates the development of AML when combined with loss of the DNA demethylase TET2. The residual PU.1 is not sufficient to overcome the aberrant methylation at PU.1 binding sites at critical myeloid enhancers (161). The acquisition of a mutation in a TET2-deficient cell that results in the loss of PU.1 expression could rapidly shift the transcriptional balance toward myeloid differentiation and cell cycle dysregulation (162, 163). Overall, reduction in PU.1 dose is a common consequence of mutations in myeloid genes, underscoring the fundamental role of a stable myeloid transcriptional network in preventing leukemogenesis.

5.6. Change in Isoform Balance

TFs often have multiple isoforms—products with different functions that arise from alternative splicing or posttranslational regulation. CEBPα is one such TF with multiple isoforms containing

distinct activities. CEBPα is required for granulocyte differentiation, and, apropos of the corollary to HITF principle 1, homozygous *Cebpa* knockout mice do not develop AML, even in the presence of a strong oncogene (73). The full-length CEBPα p42 protein contains a C-terminal basic leucine zipper DNA binding domain and an N-terminal transactivation domain, the latter of which is omitted in the shorter p30 isoform (164). Both isoforms are normally present, and their abundance is regulated translationally. As a result of this unique arrangement, different CEBPα mutations have distinct results. N-terminal mutations result in the production of p30 only, and C-terminal mutations disrupt the ability of CEBPα to bind DNA or homodimerize. Most tumors with *CEBPA* mutations have biallelic mutations of both types (165). The combination of these two mutations shifts the balance of isoforms toward p30 homodimers and away from normally predominant p42 homodimers (164). The shift to entirely p30 homodimers resulting from two N-terminal mutations is sufficient for leukemogenesis (166). However, the combination of an N-terminal mutation and a C-terminal mutation results in leukemia with faster onset, indicating C-terminal mutations might be more potent in suppressing the myeloid transcriptional network (167). These results have several implications. Importantly, biallelic mutations do not recapitulate the CEBPα null state, suggesting that there is a minimal dose of CEBPα that is required to form leukemic cells. Secondly, the mechanism by which leukemic cells alter the dose of CEBPα is partly due to an N-terminal mutation shifting the isoform balance toward the production of p30 homodimers. This later mechanism is exacerbated by further impairing p30 CEBPα activity with a C-terminal mutation. Of note, CEBPα isoforms can be deregulated through less direct means as well, as occurs with del(5q) (168).

While *RUNX1* can be inactivated, it can also be amplified by somatic or germline gains of chromosome 21, on which *RUNX1* is encoded. In TAM, a neoplasm dependent on RUNX1, the apparent gain in *RUNX1* expression through trisomy 21 actually translates into a loss of RUNX1 activity through dysregulation of the three RUNX1 isoforms. RUNX1b and RUNX1c are similar, full-length isoforms; however, RUNX1a is a primate-specific, shorter isoform that lacks the C-terminal transactivation domain (117). Compared with the other isoforms, RUNX1a has a higher affinity for DNA and is thought to act in a dominant negative manner, similar to the impact of mutations in the RUNX1 transactivation domain (169). RUNX1a expression is elevated in TAM, likely to due to the inability of the aberrant GATA1s isoform to repress RUNX1a, ultimately resulting in a decrease in wild-type RUNX1 activity (170). In addition, RUNX1a recruits other transcriptional regulators to inhibit megakaryocyte terminal differentiation and promote the proliferation of megakaryocytic blasts (170). Gain of chromosome 21 is not the only way that RUNX1 isoforms can become dysregulated. A specific mutation in a splicing factor, SRSF2[P95H], also promotes the expression of RUNX1a (171). Hence, like CEBPα, RUNX1 dose can also be altered by a change in the balance of isoforms to promote abnormal proliferation and differentiation.

5.7. Epigenetic Silencing

DNA methylation is one of many ways to inhibit the expression of genes, including HITFs. AML co-opts the DNA methylation machinery to augment tumorigenesis in concert with driver mutations, and, therefore, AML can be subdivided on the basis of DNA methylation signatures (172). In an unbiased look at diverse AMLs, GATA2 showed allele-specific expression in 60% of patients, indicating that one allele had been epigenetically silenced (173). This is particularly pronounced in the case of CEBPα[DM] AML, where 95% of cases show allele-specific expression of GATA2. In leukemias with a concomitant *GATA2* mutation, the wild-type allele is more likely to be silenced (HITF principle 1). Given the synergistic effects of *CEBPα[DM]* and *GATA2* mutation, epigenetic silencing is another route to decrease wild-type GATA2 and amplify the potency of a *GATA2* mutation. Epigenetic silencing as a mechanism of decreasing HITF dose is not limited to *GATA2* and applies to other HITFs such as *CEBPA* (174).

6. THERAPEUTIC OPTIONS

Haploinsufficiency opens at least two therapeutic windows: decreasing the activity of the residual protein to kill leukemic cells reliant on that protein or boosting the activity of the remaining wild-type protein to restore differentiation. These options can potentially avoid the toxic effects of nonspecific chemotherapy by specifically targeting cells containing the mutant allele. In an example of the first approach, knocking down the wild-type RUNX1 allele or inhibiting CBFβ binding impairs AML growth in *RUNX1* mutant xenografted mice (175). This example ought to inspire further investigation into molecules that can be given therapeutically to accomplish this goal of removing residual HITF activity in mutant cells.

Using a tetracycline-inducible (and reversible) small hairpin RNA transgene, knockdown of CUX1 expression results in de novo MDS/MPN or an alkylating agent–induced t-MN in mice (29, 95). In diseased mice, tetracycline withdrawal restores CUX1 levels and normalizes blood parameters. This example provides a genetic proof of principle for augmenting the wild-type allele as a method to treat disease driven by a HITF. Further research is warranted to ascertain methods that will allow the restoration of normal HITF protein expression.

Concordant with HITF principle 3, focal deletions and aneuploidies provide an additional opportunity to exploit the essentiality of concurrently deleted genes. Also described as collateral lethality, this concept assumes a therapeutic window for targeting cells that are haploinsufficient for an essential gene. Lenalidomide is a successful example of this. Lenalidomide and its derivatives are highly efficacious in the treatment of MDS with isolated del(5q) because lenalidomide mediates the degradation of CK1α, an essential protein encoded on 5q. Reducing CK1α levels to null results in TP53-mediated apoptosis (176). This same strategy could be applied to other recurrent chromosomal deletions in myeloid malignancies.

7. CONCLUSION

We hope to have conveyed the importance of dose in a broad sense. Traditionally, dose has been thought of diploid, haploid, and null, but many examples exist showing the pathogenic importance of non-Mendelian gene levels. In addition, there are ways to affect the dose of a HITF beyond its deletion or mutation. The myeloid cell identity is dependent on the complex transcriptional network brought about by the TFs discussed in this review, so it follows that changing the dose of one TF has secondary effects on the dose of other TFs. This balance brings added complexity to considering the genetic aberrations of a particular tumor. For example, if a patient presenting with a *TET2* mutation in the form of clonal hematopoiesis acquires another mutation that changes the dose of PU.1, there is a risk of progression to a myeloid neoplasm (161). A tumor with a *FLT3*[ITD] mutation also has an altered dose of CEBPα, and this is likely a source of the myeloid differentiation block associated with the disease. While myeloid cancers typically have fewer mutations than solid tumors, the recurrent mutations have multiple effects on the transcriptional network. A better understanding of this complexity will clarify why some patients respond to a given therapy and will open up treatment strategies for those who do not.

SUMMARY POINTS

1. A large portion of the coding genome is under genetic constraint due to haploinsufficiency, which includes almost all transcription factors (TFs) and tumor suppressor genes.

2. TFs function to influence the contact time between enhancers and target promoters to regulate the expression of target genes.

3. Loss of one allele of a gene can produce distinct effects that can be augmented by the loss of the second allele.

4. Given that myeloid neoplasms arise in cells with myeloid identity, TFs involved in myeloid cell identity are rarely mutated to zero residual activity. This is in contrast to *TP53*, the function of which is often completely lost in malignant cells.

5. There are TF doses not defined by strict Mendelian genetics that are consequential for the development of the preleukemic and leukemic states.

6. When one haploinsufficient transcription factor is altered, additional TFs are impacted due to the interwoven nature of the myeloid transcriptional network.

FUTURE ISSUES

1. What is the role of transcription factor (TF) dose in normal hematopoiesis?

2. How do TF dynamics change for hematopoietic TFs in the haploinsufficient state?

3. How do mutations in or loss of the transactivation domain and/or intrinsically disordered region (IDR) change TF dynamics and impact haploinsufficient phenotypes?

4. How do mutations in IDRs of TFs change their interaction partners and ability to interact with promoters and enhancers?

5. How do cancer cells optimize the levels of a given TF to best drive the oncogenic state, and how is this altered by environmental stresses such as inflammation and chemotherapeutic pressure?

6. How do genes concomitantly lost with haploinsufficient transcription factors (HITFs) modulate the disease phenotype in the context of a contiguous gene syndrome?

7. What mechanisms of normal TF regulation can be exploited to therapeutically manipulate the dose of a HITF to treat disease?

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Barski G, Cornefert F. 1962. Characteristics of "hybrid"-type clonal cell lines obtained from mixed cultures in vitro. *J. Natl. Cancer Inst.* 28(4):801–21

2. Harris H, Miller OJ, Klein G, Worst P, Tachibana T. 1969. Suppression of malignancy by cell fusion. *Nature* 223(5204):363–68

3. Knudson AG. 1971. Mutation and cancer: statistical study of retinoblastoma. *PNAS* 68(4):820–23

4. Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. 1998. The murine gene p27^{Kip1} is haplo-insufficient for tumour suppression. *Nature* 396(6707):177–80

5. Bartha I, di Iulio J, Venter JC, Telenti A. 2017. Human gene essentiality. *Nat. Rev. Genet.* 19(1):51–62

6. Delneri D, Hoyle DC, Gkargkas K, Cross EJM, Rash B, et al. 2007. Identification and characterization of high-flux-control genes of yeast through competition analyses in continuous cultures. *Nat. Genet.* 40(1):113–17

7. Ohnuki S, Ohya Y. 2018. High-dimensional single-cell phenotyping reveals extensive haploinsufficiency. *PLOS Biol.* 16(5):e2005130

8. White JK, Gerdin AK, Karp NA, Ryder E, Buljan M, et al. 2013. Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. *Cell* 154(2):452–64

9. **Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, et al. 2020. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581(7809):434–43**

10. Ni Z, Zhou XY, Aslam S, Niu DK. 2019. Characterization of human dosage-sensitive transcription factor genes. *Front. Genet.* 10:1208

11. Collins RL, Glessner JT, Porcu E, Lepamets M, Brandon R, et al. 2022. A cross-disorder dosage sensitivity map of the human genome. *Cell* 185(16):3041–55.e25

12. **Davoli T, Xu AW, Mengwasser KE, Sack LM, Yoon JC, et al. 2013. Cumulative haploinsufficiency and triplosensitivity drive aneuploidy patterns and shape the cancer genome. *Cell* 155(4):948–62**

13. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, et al. 2005. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* 436(7051):725–30

14. Berger AH, Knudson AG, Pandolfi PP. 2011. A continuum model for tumour suppression. *Nature* 476(7359):163–69

15. Lee JY, Nakada D, Yilmaz OH, Tothova Z, Joseph NM, et al. 2010. mTOR activation induces tumor suppressors that inhibit leukemogenesis and deplete hematopoietic stem cells after *Pten* deletion. *Cell Stem Cell* 7(5):593–605

16. Alimonti A, Carracedo A, Clohessy JG, Trotman LC, Nardella C, et al. 2010. Subtle variations in Pten dose determine cancer susceptibility. *Nat. Genet.* 42(5):454–58

17. Will B, Vogler TO, Narayanagari S, Bartholdy B, Todorova TI, et al. 2015. Minimal PU.1 reduction induces a preleukemic state and promotes development of acute myeloid leukemia. *Nat. Med.* 21(10):1172–81

18. Poluri RTK, Audet-Walsh É. 2018. Genomic deletion at *10q23* in prostate cancer: more than *PTEN* loss? *Front. Oncol.* 8:246

19. Olson OC, Kang YA, Passegué E. 2020. Normal hematopoiesis is a balancing act of self-renewal and regeneration. *Cold Spring Harb. Perspect. Med.* 10(12):a035519

20. Sanjuan-Pla A, Macaulay IC, Jensen CT, Woll PS, Luis TC, et al. 2013. Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature* 502(7470):232–36

21. Bowling S, Sritharan D, Osorio FG, Nguyen M, Cheung P, et al. 2020. An engineered CRISPR-Cas9 mouse line for simultaneous readout of lineage histories and gene expression profiles in single cells. *Cell* 181(6):1410–22.e27

22. Pei W, Shang F, Wang X, Fanti AK, Greco A, et al. 2020. Resolving fates and single-cell transcriptomes of hematopoietic stem cell clones by PolyloxExpress barcoding. *Cell Stem Cell* 27(3):383–95.e8

23. Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, Panero R, Patel SH, et al. 2018. Clonal analysis of lineage fate in native haematopoiesis. *Nature* 553(7687):212–16

24. Velten L, Haas SF, Raffel S, Blaszkiewicz S, Islam S, et al. 2017. Human haematopoietic stem cell lineage commitment is a continuous process. *Nat. Cell Biol.* 19(4):271–81

25. Lu F, Lionnet T. 2021. Transcription factor dynamics. *Cold Spring Harb. Perspect. Biol.* 13(11):a040949

26. Aly M, Ramdzan ZM, Nagata Y, Balasubramanian SK, Hosono N, et al. 2019. Distinct clinical and biological implications of *CUX1* in myeloid neoplasms. *Blood Adv.* 3(14):2164–78

9. This paper highlights the extent of genetic constraint in the human genome due to haploinsufficiency.

12. This paper demonstrates the great extent of haploinsufficiency in a pan-cancer analysis.

27. McNerney ME, Brown CD, Wang X, Bartom ET, Karmakar S, et al. 2013. *CUX1* is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. *Blood* 121(6):975–83

28. Supper E, Rudat S, Iyer V, Droop A, Wong K, et al. 2021. Cut-like homeobox1 (*CUX1*) tumor suppressor gene haploinsufficiency induces apoptosis evasion to sustain myeloid leukaemia. *Nat. Commun.* 12:2482

29. An N, Khan S, Imgruet MK, Gurbuxani SK, Konecki SN, et al. 2018. Gene dosage effect of CUX1 in a murine model disrupts HSC homeostasis and controls the severity and mortality of MDS. *Blood* 131(24):2682–97

30. Larsson AJM, Johnsson P, Hagemann-Jensen M, Hartmanis L, Faridani OR, et al. 2019. Genomic encoding of transcriptional burst kinetics. *Nature* 565(7738):251–54

31. Wheat JC, Sella Y, Willcockson M, Skoultchi AI, Bergman A, et al. 2020. Single-molecule imaging of transcription dynamics in somatic stem cells. *Nature* 583(7816):431–36

32. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, et al. 2015. Tissue-based map of the human proteome. *Science* 347(6220):1260419

33. Cook DL, Gerber AN, Tapscott SJ. 1998. Modeling stochastic gene expression: implications for haploinsufficiency. *PNAS* 95(26):15641–46

34. Kemkemer R, Schrank S, Vogel W, Gruler H, Kaufmann D. 2002. Increased noise as an effect of haploinsufficiency of the tumor-suppressor gene neurofibromatosis type 1 in vitro. *PNAS* 99(21):13783–88

35. Shaffer SM, Emert BL, Reyes Hueros RA, Cote C, Harmange G, et al. 2020. Memory sequencing reveals heritable single-cell gene expression programs associated with distinct cellular behaviors. *Cell* 182(4):947–59.e17

36. Ryl T, Kuchen EE, Bell E, Shao C, Flórez AF, et al. 2017. Cell-cycle position of single MYC-driven cancer cells dictates their susceptibility to a chemotherapeutic drug. *Cell Syst.* 5(3):237–50.e8

37. Waddington CH. 1957. *The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology*. London: Routledge. 1st ed.

38. Hansen AS, Amitai A, Cattoglio C, Tjian R, Darzacq X. 2019. Guided nuclear exploration increases CTCF target search efficiency. *Nat. Chem. Biol.* 16(3):257–66

39. Izeddin I, Récamier V, Bosanac L, Cissé II, Boudarene L, et al. 2014. Single-molecule tracking in live cells reveals distinct target-search strategies of transcription factors in the nucleus. *eLife* 3:e02230

40. Marklund E, van Oosten B, Mao G, Amselem E, Kipper K, et al. 2020. DNA surface exploration and operator bypassing during target search. *Nature* 583(7818):858–61

41. Chen J, Zhang Z, Li L, Chen BC, Revyakin A, et al. 2014. Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. *Cell* 156(6):1274–85

42. Stavreva DA, Garcia DA, Fettweis G, Gudla PR, Zaki GF, et al. 2019. Transcriptional bursting and co-bursting regulation by steroid hormone release pattern and transcription factor mobility. *Mol. Cell.* 75(6):1161–77.e11

43. Purvis JE, Karhohs KW, Mock C, Batchelor E, Loewer A, Lahav G. 2012. p53 dynamics control cell fate. *Science* 336(6087):1440–44

44. Imayoshi I, Isomura A, Harima Y, Kawaguchi K, Kori H, et al. 2013. Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. *Science* 342(6163):1203–8

45. Kull T, Wehling A, Etzrodt M, Auler M, Dettinger P, et al. 2022. NfκB signaling dynamics and their target genes differ between mouse blood cell types and induce distinct cell behavior. *Blood* 140(2):99–111

46. Natarajan A, Yardimci GG, Sheffield NC, Crawford GE, Ohler U. 2012. Predicting cell-type-specific gene expression from regions of open chromatin. *Genome Res.* 22(9):1711–22

47. Benabdallah NS, Williamson I, Illingworth RS, Kane L, Boyle S, et al. 2019. Decreased enhancer-promoter proximity accompanying enhancer activation. *Mol. Cell* 76(3):473–84.e7

48. Allahyar A, Vermeulen C, Bouwman BAM, Krijger PHL, Verstegen MJAM, et al. 2018. Enhancer hubs and loop collisions identified from single-allele topologies. *Nat. Genet.* 50(8):1151–60

49. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, et al. 2009. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. *Nat. Genet.* 42(1):53–61

50. Li J, Dong A, Saydaminova K, Chang H, Wang G, et al. 2019. Single-molecule nanoscopy elucidates RNA polymerase II transcription at single genes in live cells. *Cell* 178(2):491–506.e28

51. Mir M, Stadler MR, Ortiz SA, Hannon CE, Harrison MM, et al. 2018. Dynamic multifactor hubs interact transiently with sites of active transcription in drosophila embryos. *eLife* 7:e40497

52. Gu B, Swigut T, Spencley A, Bauer MR, Chung M, et al. 2018. Transcription-coupled changes in nuclear mobility of mammalian cis-regulatory elements. *Science* 359(6379):1050–55

53. Petrovic J, Zhou Y, Fasolino M, Goldman N, Schwartz GW, et al. 2019. Oncogenic Notch promotes long-range regulatory interactions within hyperconnected 3D cliques. *Mol. Cell* 73(6):1174–90.e12

54. Therizols P, Illingworth RS, Courilleau C, Boyle S, Wood AJ, Bickmore WA. 2014. Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. *Science* 346(6214):1238–42

55. Lundgren M, Chow CM, Sabbattini P, Georgiou A, Minaee S, Dillon N. 2000. Transcription factor dosage affects changes in higher order chromatin structure associated with activation of a heterochromatic gene. *Cell* 103(5):733–43

56. Liu J, Perumal NB, Oldfield CJ, Su EW, Uversky VN, Dunker AK. 2006. Intrinsic disorder in transcription factors. *Biochemistry* 45(22):6873–88

57. Henley MJ, Linhares BM, Morgan BS, Cierpicki T, Fierke CA, Mapp AK. 2020. Unexpected specificity within dynamic transcriptional protein-protein complexes. *PNAS* 117(44):27346–53

58. Zou H, Pan T, Gao Y, Chen R, Li S, et al. 2022. Pan-cancer assessment of mutational landscape in intrinsically disordered hotspots reveals potential driver genes. *Nucleic Acids Res.* 50(9):e499

59. Brodsky S, Jana T, Mittelman K, Chapal M, Krishna Kumar D, et al. 2020. Intrinsically disordered regions direct transcription factor in vivo binding specificity. *Mol. Cell* 79:459–71.e4

60. Ravarani CN, Erkina TY, de Baets G, Dudman DC, Erkine AM, Babu MM. 2018. High-throughput discovery of functional disordered regions: investigation of transactivation domains. *Mol. Syst. Biol.* 14(5):8190

61. Boija A, Klein IA, Sabari BR, Dall'Agnese A, Coffey EL, et al. 2018. Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell* 175(7):1842–55.e16

62. Chong S, Dugast-Darzacq C, Liu Z, Dong P, Dailey GM, et al. 2018. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* 361(6400):eaar2555

63. Sabari BR, Dall'Agnese A, Boija A, Klein IA, Coffey EL, et al. 2018. Coactivator condensation at super-enhancers links phase separation and gene control. *Science* 361(6400):eaar3958

64. Furlong EEM, Levine M. 2018. Developmental enhancers and chromosome topology. *Science* 361(6409):1341–45

65. Cho WK, Spille JH, Hecht M, Lee C, Li C, et al. 2018. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* 361(6400):412–15

66. Deviri D, Safran SA. 2021. Physical theory of biological noise buffering by multicomponent phase separation. *PNAS* 118(25):e2100099118

67. Ng KP, Hu Z, Ebrahem Q, Negrotto S, Lausen J, Saunthararajah Y. 2013. Runx1 deficiency permits granulocyte lineage commitment but impairs subsequent maturation. *Oncogenesis* 2(11):e78

68. Sinha T, Lammerts van Bueren K, Dickel DE, Zlatanova I, Thomas R, et al. 2022. Differential Etv2 threshold requirement for endothelial and erythropoietic development. *Cell Rep.* 39(9):110881

69. Giorgetti L, Siggers T, Tiana G, Caprara G, Notarbartolo S, et al. 2010. Noncooperative interactions between transcription factors and clustered DNA binding sites enable graded transcriptional responses to environmental inputs. *Mol. Cell* 37(3):418–28

70. Staber PB, Zhang P, Ye M, Welner RS, Nombela-Arrieta C, et al. 2013. Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells. *Mol. Cell* 49(5):934–46

71. Naqvi S, Kim S, Hoskens H, Matthews HS, Spritz RA, et al. 2023. Precise modulation of transcription factor levels identifies features underlying dosage sensitivity. *Nat. Genet.* 55:841–51

72. Staber PB, Zhang P, Ye M, Welner RS, Levantini E, et al. 2014. The Runx-PU.1 pathway preserves normal and AML/ETO9a leukemic stem cells. *Blood* 124(15):2391–99

73. Ye M, Zhang H, Yang H, Koche R, Staber PB, et al. 2015. Hematopoietic differentiation is required for initiation of acute myeloid leukemia. *Cell Stem Cell* 17(5):611–23

55. This paper demonstrates how monoallelic loss of a HITF can influence the nuclear position of target genes.

71. This paper represents the most advanced analysis of the mechanism of a HITF using a novel degron system.

73. This paper demonstrates the requirement of myeloid cell identity for the development of myeloid neoplasm.

74. Huang N, Lee I, Marcotte EM, Hurles ME. 2010. Characterising and predicting haploinsufficiency in the human genome. *PLOS Genet*. 6(10):e1001154

75. Assi SA, Imperato MR, Coleman DJL, Pickin A, Potluri S, et al. 2018. Subtype-specific regulatory network rewiring in acute myeloid leukemia. *Nat. Genet*. 51(1):151–62

76. Jotte MRM, McNerney ME. 2022. The significance of CUX1 and chromosome 7 in myeloid malignancies. *Curr. Opin. Hematol*. 29(2):92–102

77. Ben-David U, Amon A. 2019. Context is everything: aneuploidy in cancer. *Nat. Rev. Genet*. 21(1):44–62

78. Gao T, Ptashkin R, Bolton KL, Sirenko M, Fong C, et al. 2021. Interplay between chromosomal alterations and gene mutations shapes the evolutionary trajectory of clonal hematopoiesis. *Nat. Commun*. 12:338

79. Chunduri NK, Menges P, Zhang X, Wieland A, Gotsmann VL, et al. 2021. Systems approaches identify the consequences of monosomy in somatic human cells. *Nat. Commun*. 12:5576

80. Rücker FG, Schlenk RF, Bullinger L, Kayser S, Teleanu V, et al. 2012. *TP53* alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood* 119(9):2114–21

81. Beach RR, Ricci-Tam C, Brennan CM, Silberman RE, Springer M, Amon A. 2017. Aneuploidy causes non-genetic individuality. *Cell* 169:229–42

82. Farquhar KS, Charlebois DA, Szenk M, Cohen J, Nevozhay D, Balázsi G. 2019. Role of network-mediated stochasticity in mammalian drug resistance. *Nat. Commun*. 10:2766

83. Schukken KM, Sheltzer JM. 2022. Extensive protein dosage compensation in aneuploid human cancers. *Genome Res*. 32(7):1254–70

84. Liu Y, Chen C, Xu Z, Scuoppo C, Rillahan CD, et al. 2016. Deletions linked to *TP53* loss drive cancer through p53-independent mechanisms. *Nature* 531(7595):471–75

85. Stoddart A, Nakitandwe J, Chen SC, Downing JR, Le Beau MM. 2015. Haploinsufficient loss of multiple 5q genes may fine-tune Wnt signaling in del(5q) therapy-related myeloid neoplasms. *Blood* 126(26):2899–901

86. Joslin JM, Fernald AA, Tennant TR, Davis EM, Kogan SC, et al. 2007. Haploinsufficiency of *EGR1*, a candidate gene in the del(5q), leads to the development of myeloid disorders. *Blood* 110(2):719–26

87. Stoddart A, Fernald AA, Davis EM, McNerney ME, le Beau MM. 2022. *EGR1* haploinsufficiency confers a fitness advantage to hematopoietic stem cells following chemotherapy. *Exp Hematol*. 115:54–67

88. Stoddart A, Fernald AA, Wang J, Davis EM, Karrison T, et al. 2014. Haploinsufficiency of del(5q) genes, *Egr1* and *Apc*, cooperate with *Tp53* loss to induce acute myeloid leukemia in mice. *Blood* 123(7):1069–78

89. Pitsouli C, Perrimon N. 2013. The homeobox transcription factor cut coordinates patterning and growth during *Drosophila* airway remodeling. *Sci. Signal*. 6(263):ra12

90. Grueber WB, Jan LY, Jan YN. 2003. Different levels of the homeodomain protein cut regulate distinct dendrite branching patterns of *Drosophila* multidendritic neurons. *Cell* 112(6):805–18

91. Arthur RK, An N, Khan S, McNerney ME. 2017. The haploinsufficient tumor suppressor, CUX1, acts as an analog transcriptional regulator that controls target genes through distal enhancers that loop to target promoters. *Nucleic Acids Res*. 45(11):6350–61

92. Wong CC, Martincorena I, Rust AG, Rashid M, Alifrangis C, et al. 2014. Inactivating *CUX1* mutations promote tumorigenesis. *Nat. Genet*. 46(1):33–38

93. Schwartz JR, Ma J, Lamprecht T, Walsh M, Wang S, et al. 2017. The genomic landscape of pediatric myelodysplastic syndromes. *Nat. Commun*. 8:1557

94. Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, et al. 2003. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood* 102(1):43–52

95. Imgruet MK, Lutze J, An NN, Hu B, Khan S, et al. 2021. Loss of a 7q gene, *CUX1*, disrupts epigenetic-driven DNA repair and drives therapy-related myeloid neoplasms. *Blood* 138:790–805

96. Baker SJ, Ma'ayan A, Lieu YK, John P, Reddy MVR, et al. 2014. B-*myb* is an essential regulator of hematopoietic stem cell and myeloid progenitor cell development. *PNAS* 111(8):3122–27

97. Clarke M, Dumon S, Ward C, Jäger R, Freeman S, et al. 2012. *MYBL2* haploinsufficiency increases susceptibility to age-related haematopoietic neoplasia. *Leukemia* 27(3):661–70

98. Heinrichs S, Conover LF, Bueso-Ramos CE, Kilpivaara O, Stevenson K, et al. 2013. *MYBL2* is a sub-haploinsufficient tumor suppressor gene in myeloid malignancy. *eLife* 2:e00825

99. Braulke F, Müller-Thomas C, Götze K, Platzbecker U, Germing U, et al. 2015. Frequency of del(12p) is commonly underestimated in myelodysplastic syndromes: results from a German diagnostic study in comparison with an international control group. *Genes Chromosomes Cancer* 54(12):809–17

100. Feurstein S, Rücker FG, Bullinger L, Hofmann W, Manukjan G, et al. 2014. Haploinsufficiency of *ETV6* and *CDKN1B* in patients with acute myeloid leukemia and complex karyotype. *BMC Genom.* 151:784

101. Green SM, Coyne HJ, McIntosh LP, Graves BJ. 2010. DNA binding by the ETS protein TEL (ETV6) is regulated by autoinhibition and self-association. *J. Biol. Chem.* 285(24):18496–504

102. Churpek JE, Bresnick EH. 2019. Transcription factor mutations as a cause of familial myeloid neoplasms. *J. Clin. Investig.* 129(2):476–88

103. Fisher MH, Kirkpatrick GD, Stevens B, Jones C, Callaghan M, et al. 2020. *ETV6* germline mutations cause HDAC3/NCOR2 mislocalization and upregulation of interferon response genes. *JCI Insight* 5(18):e140332

104. Bernard E, Nannya Y, Hasserjian RP, Devlin SM, Tuechler H, et al. 2020. Implications of *TP53* allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. *Nat. Med.* 26(10):1549–56

105. Boettcher S, Miller PG, Sharma R, McConkey M, Leventhal M, et al. 2019. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. *Science* 365(6453):599–604

106. Venkatachalam S, Shi YP, Jones SN, Vogel H, Bradley A, et al. 1998. Retention of wild-type p53 in tumors from p53 heterozygous mice: Reduction of p53 dosage can promote cancer formation. *EMBO J.* 17(16):4657–67

107. Solimini NL, Xu Q, Mermel CH, Liang AC, Schlabach MR, et al. 2012. Recurrent hemizygous deletions in cancers may optimize proliferative potential. *Science* 336(6090):104–9

108. Davoli T, Uno H, Wooten EC, Elledge SJ. 2017. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* 355(6322):eaaf8399

109. Fischer K, Fröhling S, Scherer SW, Brown JMA, Scholl C, et al. 1997. Molecular cytogenetic delineation of deletions and translocations involving chromosome band 7q22 in myeloid leukemias. *Blood* 89(6):2036–41

110. Wong JC, Weinfurtner KM, del pilar Alzamora M, Kogan SC, Burgess MR, et al. 2015. Functional evidence implicating chromosome 7q22 haploinsufficiency in myelodysplastic syndrome pathogenesis. *eLife* 4:e07839

111. Ouillette P, Collins R, Shakhan S, Li J, Li C, et al. 2011. The prognostic significance of various 13q14 deletions in chronic lymphocytic leukemia. *Clin. Cancer Res.* 17(21):6778–90

112. Rego EM, Wang ZG, Peruzzi D, He LZ, Cordon-Cardo C, Pandolfi PP. 2001. Role of promyelocytic leukemia (Pml) protein in tumor suppression. *J. Exp. Med.* 193(4):521–30

113. Welch JS, Klco JM, Varghese N, Nagarajan R, Ley TJ. 2011. Rara haploinsufficiency modestly influences the phenotype of acute promyelocytic leukemia in mice. *Blood* 117(8):2460–68

114. Gröschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BAM, et al. 2014. A single oncogenic enhancer rearrangement causes concomitant *EVI1* and *GATA2* deregulation in leukemia. *Cell* 157(2):369–81

115. Katayama S, Suzuki M, Yamaoka A, Keleku-Lukwete N, Katsuoka F, et al. 2017. GATA2 haploinsufficiency accelerates EVI1-driven leukemogenesis. *Blood* 130(7):908–19

116. Gröschel S, Sanders MA, Hoogenboezem R, Zeilemaker A, Havermans M, et al. 2015. Mutational spectrum of myeloid malignancies with inv(3)/t(3;3) reveals a predominant involvement of RAS/RTK signaling pathways. *Blood* 125(1):133–39

117. de Bruijn M, Dzierzak E. 2017. Runx transcription factors in the development and function of the definitive hematopoietic system. *Blood* 129(15):2061–69

118. Yan M, Burel SA, Peterson LF, Kanbe E, Iwasaki H, et al. 2004. Deletion of an AML1-ETO C-terminal NcoR/SMRT-interacting region strongly induces leukemia development. *PNAS* 101(49):17186–91

119. Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, et al. 2001. AML1-ETO downregulates the granulocytic differentiation factor C/EBPα in t(8;21) myeloid leukemia. *Nat. Med.* 7(4):444–51

120. Choi Y, Elagib KE, Delehanty LL, Goldfarb AN. 2006. Erythroid inhibition by the leukemic fusion AML1-ETO is associated with impaired acetylation of the major erythroid transcription factor GATA-1. *Cancer Res.* 66(6):2990–96

121. Vangala RK, Heiss-Neumann MS, Rangatia JS, Singh SM, Schoch C, et al. 2003. The myeloid master regulator transcription factor PU.1 is inactivated by AML1-ETO in t(8;21) myeloid leukemia. *Blood* 101(1):270–77

122. Rubin C, Larson R, Anastasi J, Winter J, Thangavelu M, et al. 1990. t(3;21)(q26;q22): a recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. *Blood* 76(12):2594–98

123. Loke J, Assi SA, Imperato MR, Ptasinska A, Cauchy P, et al. 2017. RUNX1-ETO and RUNX1-EVI1 differentially reprogram the chromatin landscape in t(8;21) and t(3;21) AML. *Cell Rep.* 19(8):1654–68

124. Ptasinska A, Assi SA, Martinez-Soria N, Imperato MR, Piper J, et al. 2014. Identification of a dynamic core transcriptional network in t(8;21) AML that regulates differentiation block and self-renewal. *Cell Rep.* 8(6):1974–88

125. Ben-Ami O, Friedman D, Leshkowitz D, Goldenberg D, Orlovsky K, et al. 2013. Addiction of t(8;21) and inv(16) acute myeloid leukemia to native RUNX1. *Cell Rep.* 4(6):1131–43

126. Lukasik SM, Zhang L, Corpora T, Tomanicek S, Li Y, et al. 2002. Altered affinity of CBFβ-SMMHC for Runx1 explains its role in leukemogenesis. *Nat. Struct. Biol.* 9(9):674–79

127. Zhen T, Cao Y, Ren G, Zhao L, Hyde RK, et al. 2020. RUNX1 and CBFβ-SMMHC transactivate target genes together in abnormal myeloid progenitors for leukemia development. *Blood* 136(21):2373–85

128. Hyde RK, Zhao L, Alemu L, Liu PP. 2015. Runx1 is required for hematopoietic defects and leukemogenesis in Cbfb-MYH11 knock-in mice. *Leukemia* 29(8):1771–78

129. Qi J, Singh S, Hua WK, Cai Q, Chao SW, et al. 2015. HDAC8 inhibition specifically targets inv(16) acute myeloid leukemic stem cells by restoring p53 acetylation. *Cell Stem Cell* 17(5):597–610

130. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, et al. 2016. Genomic classification and prognosis in acute myeloid leukemia. *N. Engl. J. Med.* 374(23):2209–21

131. Sakurai M, Kunimoto H, Watanabe N, Fukuchi Y, Yuasa S, et al. 2014. Impaired hematopoietic differentiation of RUNX1-mutated induced pluripotent stem cells derived from FPD/AML patients. *Leukemia* 28(12):2344–54

132. Estevez B, Borst S, Jarocha D, Sudunagunta V, Gonzalez M, et al. 2021. RUNX-1 haploinsufficiency causes a marked deficiency of megakaryocyte-biased hematopoietic progenitor cells. *Blood* 137(19):2662–75

133. Bellissimo DC, Speck NA. 2017. RUNX1 mutations in inherited and sporadic leukemia. *Front. Cell Dev. Biol.* 5:111

134. Antony-Debré I, Manchev VT, Balayn N, Bluteau D, Tomowiak C, et al. 2015. Level of RUNX1 activity is critical for leukemic predisposition but not for thrombocytopenia. *Blood* 125:930–40

135. Tsai SC, Shih LY, Liang ST, Huang YJ, Kuo MC, et al. 2015. Biological activities of RUNX1 mutants predict secondary acute leukemia transformation from chronic myelomonocytic leukemia and myelodysplastic syndromes. *Clin. Cancer Res.* 21(15):3541–51

136. McDevitt MA, Shivdasani RA, Fujiwara Y, Yang H, Orkin SH. 1997. A "knockdown" mutation created by cis-element gene targeting reveals the dependence of erythroid cell maturation on the level of transcription factor GATA-1. *PNAS* 94(13):6781–85

137. Gregory T, Yu C, Ma A, Orkin SH, Blobel GA, Weiss MJ. 1999. GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-x_L expression. *Blood* 94(1):87–96

138. Yoshida K, Toki T, Okuno Y, Kanezaki R, Shiraishi Y, et al. 2013. The landscape of somatic mutations in Down syndrome-related myeloid disorders. *Nat. Genet.* 45(11):1293–99

139. Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, et al. 2002. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat. Genet.* 32(1):148–52

140. Rodrigues NP, Janzen V, Forkert R, Dombkowski DM, Boyd AS, et al. 2005. Haploinsufficiency of GATA-2 perturbs adult hematopoietic stem-cell homeostasis. *Blood* 106(2):477–84

141. Persons DA, Allay JA, Allay ER, Ashmun RA, Orlic D, et al. 1999. Enforced expression of the GATA-2 transcription factor blocks normal hematopoiesis. *Blood* 93(2):488–99

142. Collin M, Dickinson R, Bigley V. 2015. Haematopoietic and immune defects associated with GATA2 mutation. *Br. J. Haematol.* 169(2):173–87

143. Ping N, Sun A, Song Y, Wang Q, Yin J, et al. 2016. Exome sequencing identifies highly recurrent somatic *GATA2* and *CEBPA* mutations in acute erythroid leukemia. *Leukemia* 31(1):195–202

144. di Genua C, Valletta S, Buono M, Stoilova B, Sweeney C, et al. 2020. C/EBPα and GATA-2 mutations induce bilineage acute erythroid leukemia through transformation of a neomorphic neutrophil-erythroid progenitor. *Cancer Cell* 37(5):690–704.e8

145. Sperling AS, Guerra VA, Kennedy JA, Yan Y, Hsu JI, et al. 2022. Lenalidomide promotes the development of *TP53*-mutated therapy-related myeloid neoplasms. *Blood* 140(16):1753–63

146. Chen S, Wang Q, Yu H, Capitano ML, Vemula S, et al. 2019. Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway. *Nat. Commun.* 101:5649

147. Loizou E, Banito A, Livshits G, Ho YJ, Koche RP, et al. 2019. A gain-of-function p53-mutant oncogene promotes cell fate plasticity and myeloid leukemia through the pluripotency factor FOXH1. *Cancer Discov.* 9(7):962–79

148. Iwasaki H, Somoza C, Shigematsu H, Duprez EA, Iwasaki-Arai J, et al. 2005. Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. *Blood* 106(5):1590–600

149. Scott EW, Simon MC, Anastasi J, Singh H. 1994. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science* 265(5178):1573–77

150. Rosenbauer F, Wagner K, Kutok JL, Iwasaki H, le Beau MM, et al. 2004. Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. *Nat. Genet.* 36(6):624–30

151. Huang G, Zhang P, Hirai H, Elf S, Yan X, et al. 2007. PU.1 is a major downstream target of AML1 (RUNX1) in adult mouse hematopoiesis. *Nat. Genet.* 40(1):51–60

152. Trinh BQ, Ummarino S, Zhang Y, Ebralidze AK, Bassal MA, et al. 2021. Myeloid lncRNA *LOUP* mediates opposing regulatory effects of RUNX1 and RUNX1-ETO in t(8;21) AML. *Blood* 138(15):1331–44

153. van der Kouwe E, Heller G, Czibere A, Pulikkan JA, Agreiter C, et al. 2021. Core-binding factor leukemia hijacks the T-cell-prone PU.1 antisense promoter. *Blood* 138(15):1345–58

154. Huang G, Zhao X, Wang L, Elf S, Xu H, et al. 2011. The ability of MLL to bind RUNX1 and methylate H3K4 at PU.1 regulatory regions is impaired by MDS/AML-associated RUNX1/AML1 mutations. *Blood* 118(25):6544–52

155. Mueller BU, Pabst T, Fos J, Petkovic V, Fey MF, et al. 2006. ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression. *Blood* 107(8):3330–38

156. Mizuki M, Schwäble J, Steur C, Choudhary C, Agrawal S, et al. 2003. Suppression of myeloid transcription factors and induction of STAT response genes by AML-specific Flt3 mutations. *Blood* 101(8):3164–73

157. Yun H, Narayan N, Vohra S, Giotopoulos G, Mupo A, et al. 2021. Mutational synergy during leukemia induction remodels chromatin accessibility, histone modifications and three-dimensional DNA topology to alter gene expression. *Nat. Genet.* 53(10):1443–55

158. Yeamans C, Wang D, Paz-Priel I, Torbett BE, Tenen DG, Friedman AD. 2007. C/EBPα binds and activates the PU.1 distal enhancer to induce monocyte lineage commitment. *Blood* 110(9):3136–42

159. Gu X, Ebrahem Q, Mahfouz RZ, Hasipek M, Enane F, et al. 2018. Leukemogenic nucleophosmin mutation disrupts the transcription factor hub that regulates granulomonocytic fates. *J. Clin. Investig.* 128(10):4260–79

160. Pianigiani G, Betti C, Bigerna B, Rossi R, Brunetti L. 2020. PU.1 subcellular localization in acute myeloid leukaemia with mutated *NPM1*. *Br. J. Haematol.* 188(1):184–87

161. Aivalioti MM, Bartholdy BA, Pradhan K, Bhagat TD, Zintiridou A, et al. 2022. PU.1-dependent enhancer inhibition separates *Tet2*-deficient hematopoiesis from malignant transformation. *Blood Cancer Discov.* 3(5):444–67

162. Kaasinen E, Kuismin O, Rajamäki K, Ristolainen H, Aavikko M, et al. 2019. Impact of constitutional *TET2* haploinsufficiency on molecular and clinical phenotype in humans. *Nat. Commun.* 101:1252

161. This paper demonstrates the importance of minor changes in TF dose for the progression of leukemia.

163. Tulstrup M, Soerensen M, Hansen JW, Gillberg L, Needhamsen M, et al. 2021. *TET2* mutations are associated with hypermethylation at key regulatory enhancers in normal and malignant hematopoiesis. *Nat. Commun.* 121:6061

164. Ohlsson E, Schuster MB, Hasemann M, Porse BT. 2015. The multifaceted functions of C/EBPα in normal and malignant haematopoiesis. *Leukemia* 30(4):767–75

165. Mannelli F, Ponziani V, Bencini S, Bonetti MI, Benelli M, et al. 2017. *CEBPA*-double-mutated acute myeloid leukemia displays a unique phenotypic profile: a reliable screening method and insight into biological features. *Haematologica* 102(3):529–40

166. Kirstetter P, Schuster MB, Bereshchenko O, Moore S, Dvinge H, et al. 2008. Modeling of C/EBPα mutant acute myeloid leukemia reveals a common expression signature of committed myeloid leukemia-initiating cells. *Cancer Cell* 13(4):299–310

167. Bereshchenko O, Mancini E, Moore S, Bilbao D, Månsson R, et al. 2009. Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPα mutant AML. *Cancer Cell* 16(5):390–400

168. Khanna-Gupta A, Abayasekara N, Levine M, Sun H, Virgilio M, et al. 2012. Up-regulation of translation eukaryotic initiation factor 4E in nucleophosmin 1 haploinsufficient cells results in changes in CCAAT enhancer-binding protein α activity: implications in myelodysplastic syndrome and acute myeloid leukemia. *J. Biol. Chem.* 287(39):32728–37

169. Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, et al. 1995. An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. *EMBO J.* 14(2):341–50

170. Gialesaki S, Bräuer-Hartmann D, Bhayadia R, Alejo-Valle O, Issa H, et al. 2022. *RUNX1* isoform disequilibrium in the development of trisomy 21 associated myeloid leukemia. bioRxiv 2022.03.07.483334. https://doi.org/10.1101/2022.03.07.483334

171. Sakurai H, Harada Y, Ogata Y, Kagiyama Y, Shingai N, et al. 2017. Overexpression of *RUNX1* short isoform has an important role in the development of myelodysplastic/myeloproliferative neoplasms. *Blood Adv.* 1(18):1382–86

172. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, et al. 2010. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. *Cancer Cell* 17(1):13–27

173. Mulet-Lazaro R, van Herk S, Erpelinck C, Bindels E, Sanders MA, et al. 2021. Allele-specific expression of *GATA2* due to epigenetic dysregulation in *CEBPA* double-mutant AML. *Blood* 138(2):160–77

174. Lin TC, Hou HA, Chou WC, Ou DL, Yu SL, et al. 2010. *CEBPA* methylation as a prognostic biomarker in patients with de novo acute myeloid leukemia. *Leukemia* 25(1):32–40

175. Mill CP, Fiskus W, DiNardo CD, Qian Y, Raina K, et al. 2019. RUNX1-targeted therapy for AML expressing somatic or germline mutation in RUNX1. *Blood* 134(1):59–73

176. Krönke J, Fink EC, Hollenbach PW, MacBeth KJ, Hurst SN, et al. 2015. Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. *Nature* 523(7559):183–88

177. Pich O, Reyes-Salazar I, Gonzalez-Perez A, Lopez-Bigas N. 2022. Discovering the drivers of clonal hematopoiesis. *Nat. Commun.* 131:4267

178. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, et al. 2014. Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* 371(26):2488–98

179. Bolton KL, Koh Y, Foote MB, Im H, Jee J, et al. 2021. Clonal hematopoiesis is associated with risk of severe Covid-19. *Nat. Commun.* 121:5975

180. Gallì A, Todisco G, Catamo E, Sala C, Elena C, et al. 2021. Relationship between clone metrics and clinical outcome in clonal cytopenia. *Blood* 138(11):965–76

181. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, et al. 2002. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. *Blood* 99(3):840–49

182. Such E, Cervera J, Costa D, Solé F, Vallespí T, et al. 2011. Cytogenetic risk stratification in chronic myelomonocytic leukemia. *Haematologica* 96(3):375–83

183. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, et al. 2013. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 122(22):3616–27

184. Grinfeld J, Nangalia J, Baxter EJ, Wedge DC, Angelopoulos N, et al. 2018. Classification and personalized prognosis in myeloproliferative neoplasms. *N. Engl. J. Med.* 379(15):1416–30

170. This paper demonstrates how gain of a chromosome can negatively affect the dose of a TF.

175. This paper highlights one possible treatment option for neoplasms dependent on a HITF.

185. Marcellino BK, Hoffman R, Tripodi J, Lu M, Kosiorek H, et al. 2018. Advanced forms of MPNs are accompanied by chromosomal abnormalities that lead to dysregulation of TP53. *Blood Adv.* 2(24):3581–89

186. Yoshizato T, Nannya Y, Atsuta Y, Shiozawa Y, Iijima-Yamashita Y, et al. 2017. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. *Blood* 129(17):2347–58

187. Bernard E, Tuechler H, Greenberg PL, Hasserjian RP, Ossa JEA, et al. 2022. Molecular International Prognostic Scoring System for myelodysplastic syndromes. *NEJM Evid.* 1(7). **https://doi.org/10.1056/EVIDoa2200008**

188. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, et al. 2011. Clinical effect of point mutations in myelodysplastic syndromes. *N. Engl. J. Med.* 364(26):2496–506

189. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, et al. 2001. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood* 98(5):1312–20

190. Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, et al. 2018. Functional genomic landscape of acute myeloid leukaemia. *Nature* 562(7728):526–31

191. Fröhling S, Schlenk RF, Kayser S, Morhardt M, Benner A, et al. 2006. Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. *Blood* 108(10):3280–88

192. Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. 2013. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N. Engl. J. Med.* 368(22):2059–74

193. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, et al. 2015. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* 125(9):1367–76

Contents

Errata

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