Therapy-related myeloid neoplasms: when genetics and environment collide

Megan E. McNerney[1,3], Lucy A. Godley[2,3] and Michelle M. Le Beau[2,3]

Abstract | Therapy-related myeloid neoplasms (t-MN) arise as a late effect of chemotherapy and/or radiation administered for a primary condition, typically a malignant disease, solid organ transplant or autoimmune disease. Survival is measured in months, not years, making t-MN one of the most aggressive and lethal cancers. In this Review, we discuss recent developments that reframe our understanding of the genetic and environmental aetiology of t-MN. Emerging data are illuminating who is at highest risk of developing t-MN, why t-MN are chemoresistant and how we may use this information to treat and ultimately prevent this lethal disease.

Acute myeloid leukaemia (AML). A cancer of the myeloid lineage of haematopoietic cells associated with an expansion of immature cells (≥20% blasts) and defective differentiation into the mature myeloid lineages.

Myelodysplastic syndrome (MDS). A group of clonal disorders with dysfunctional and dysplastic haematopoiesis of one or more myeloid lineage(s) leading to decreased maturation of normal myeloid cells with <20% blasts and a risk of leukaemic transformation.

[1]*Department of Pathology and the Department of Pediatrics, The University of Chicago, Chicago, Illinois 60637, USA.*
[2]*Department of Medicine, The University of Chicago, Chicago, Illinois 60637, USA.*
[3]*University of Chicago Medicine Comprehensive Cancer Center, Chicago, Illinois 60637, USA.*

Correspondence to M.M.L.B.
mlebeau@bsd.uchicago.edu

doi:10.1038/nrc.2017.60
Published online 24 Aug 2017

Therapy-related myeloid neoplasms (t-MN), which comprise therapy-related acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN), are a late complication of cytotoxic therapy — chemotherapy and/or radiation therapy — used in the treatment of both malignant and non-malignant diseases[1]. There are several clinical subsets of t-MN that correlate with the nature of the prior therapy. The most common subtype (in ~70% of patients) is characterized by loss of part of chromosome 5 (del(5q)) and/or either part or all of chromosome 7 (del(7q) or –7, respectively) and is characteristic of patients who received alkylating agents and/or radiation therapy. Clinically, this subtype has a long latency period (on average five years), presents as MDS, which often progresses rapidly to AML with multilineage dysplasia, and is associated with a poor prognosis (median survival: 8 months). A second major subset of t-MN is seen in patients who received topoisomerase II inhibitors. Clinically, this subtype has a shorter latency period (in the range of 1 to 2 years), presents as overt leukaemia without an antecedent MDS and is associated with a more favourable response to intensive induction therapy. Translocations involving histone-lysine *N*-methyltransferase 2A (*KMT2A*; also known as *MLL*) at 11q23.3 or runt-related transcription factor 1 (*RUNX1*; also known as *AML1*) at 21q22.1 are common in this subgroup.

Historically, t-MN has been considered a consequence of DNA damage induced by cytotoxic therapy in normal haematopoietic stem or progenitor cells. There is currently active debate in the field of cancer biology regarding the relative contributions of inherited risk factors, environmental exposures and stochastic events in the aetiology of cancer. One study estimated that extrinsic factors contribute to 70–90% of the risk in most cancer types[2]. By definition, therapy-related cancers, also known as second neoplasms, have a strong extrinsic driver (that is, previous chemotherapy and/or radiotherapy). Nonetheless, up to 10% of patients have had more than one previous cancer before their diagnosis of t-MN; thus, the concept that exposure to therapeutic agents is the sole cause of t-MN has been unsatisfactory and raises multiple questions. Such questions include why some people develop t-MN and others do not, why second cancers have a latency of ten years or more in some patients and why t-MN are inherently chemoresistant.

Technological advances such as next-generation sequencing (NGS) have transformed the cancer field. We have learned that 8.5–12.6% of cancer patients have an inherited mutation in a cancer-associated gene compared with 1–2.7% of individuals without cancer[3–5]. As discussed below, this frequency is likely to be much higher in t-MN patients. Studies are also coalescing that implicate a role for haematopoietic stem cells (HSCs) with acquired somatic mutations existing before treatment of the primary disease and that these HSCs are precursors for t-MN. This knowledge of inherited and pretreatment somatic genetic alterations sheds light on t-MN aetiology and could potentially help identify the long-awaited predictors of who is at risk of developing t-MN.

In this Review, we synthesize recent findings that are reframing our understanding of t-MN pathogenesis. We highlight new experimental data that challenge prevailing beliefs regarding t-MN aetiology and show that chemotherapy and/or radiotherapy promotes clonal selection of pre-existing mutant HSCs, in addition to inducing leukaemogenic mutations directly[6]; t-MNs are genetically and aetiologically similar to other high-risk myeloid neoplasms[6–8]; large chromosomal deletions do not harbour a single, recessive tumour suppressor gene

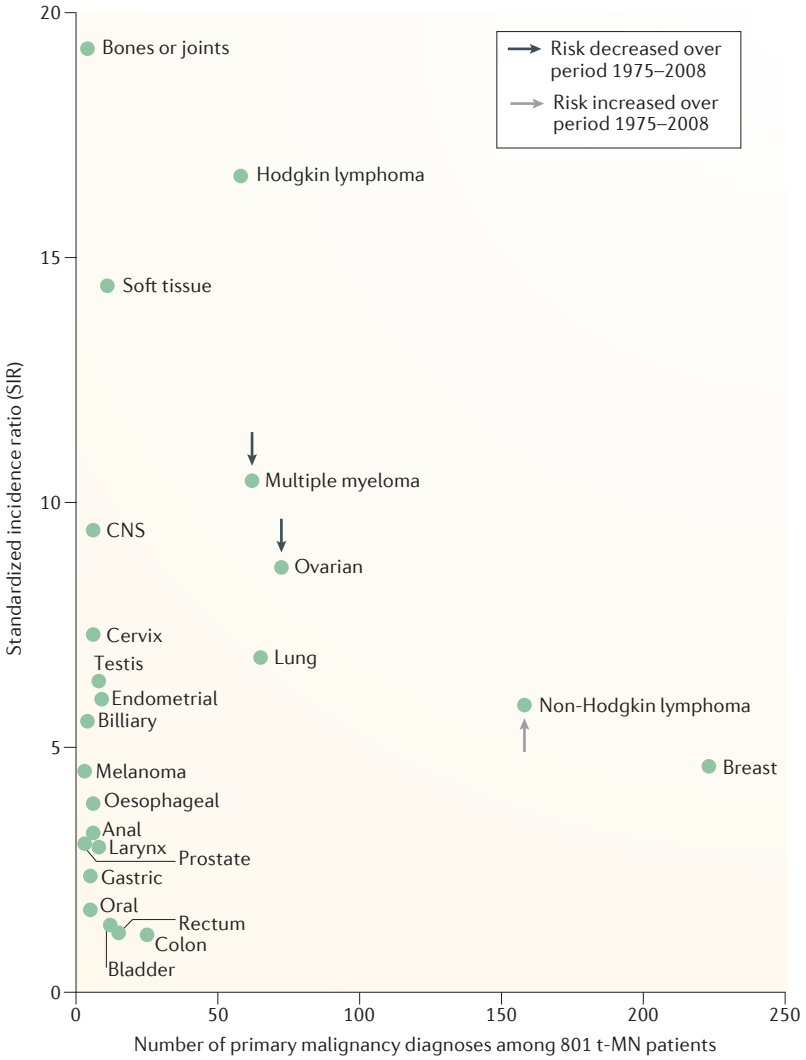


Figure 1 | **Risk of therapy-related myeloid neoplasms after chemotherapy in the United States from 1975 to 2008.** Among adults treated with chemotherapy for a primary malignancy, 801 patients with a therapy-related myeloid neoplasm (t-MN) were identified from Surveillance, Epidemiology and End Results (SEER)[21]. The number of cases and standardized incidence ratio (SIR) for t-MN are shown by primary tumour type. The risk of t-MN has decreased over time for patients with multiple myeloma or ovarian cancer (black arrows), whereas the risk has increased for patients with non-Hodgkin lymphoma (grey arrow). CNS, central nervous system.

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN). Clonal haematopoietic malignancy with features of MDS and excess production of one or more myeloid lineages.

Contiguous gene syndrome (CGS). Genetic disorder caused by chromosomal copy number change, leading to combined dosage imbalance of multiple neighbouring genes typically on the scale of <5 Mb.

but rather are part of a contiguous gene syndrome (CGS) invoking haploinsufficiency[9–11] and an aberrant bone marrow microenvironment contributes to malignant haematopoiesis[12–15]. The models described here are not mutually exclusive, and one or multiple different pathways may contribute to these malignancies in any individual patient. Moreover, these paradigms may also apply to other cancers that arise from the interaction between genetics and environment.

Epidemiology

In 2012, there were an estimated 13.7 million cancer survivors in the United States[16]. An ageing population, a commensurate increase in the number of individuals afflicted with cancer, and improvements in oncology care are anticipated to lead to 18 million cancer survivors within a decade[16,17]. Although progress in treating cancer is welcome, it also creates a need to attend to the long-term health risks of survivors. The incidence of t-MN — currently 0.62 per 100,000 men and women (which accounts for ~10–20% of newly diagnosed cases of AML or MDS[18,19]) — is expected to rise in parallel with the increase in the number of cancer survivors worldwide. The median age of individuals diagnosed with t-MN is 64 years, but the disease can occur at any age[20]. Chemotherapy treatment increases the incidence of t-MN by 4.7-fold[21], and a younger age at the time of exposure further increases that risk[21,22]. For example, the standardized incidence ratio (SIR) for t-MN is higher for patients treated with chemotherapy for breast cancer at 20–40 years of age compared with those over 60 years of age[21]. The relationship between age at exposure to radiation and risk of t-MN is less clear[23].

Treatment for most primary tumour types carries a risk of t-MN (FIG. 1). t-MN is rare, although it can occur with high frequency in certain types of cancer. The most common primary malignancies with an associated risk are breast cancer and haematological malignancies (particularly non-Hodgkin lymphoma, Hodgkin lymphoma and multiple myeloma)[21,23,24]. Patients with breast cancer in the United States have a 0.5% cumulative 10-year incidence of t-MN[25], and patients with Hodgkin lymphoma have a cumulative 6-year incidence of 0.9%[26]. Depending on the treatment regimen, up to 10% of patients with non-Hodgkin lymphoma[27], 8.2% of patients with chronic lymphocytic leukaemia (CLL)[28] and 3.4% of patients with multiple myeloma[29] develop t-MN. Patients with a prior bone or soft-tissue sarcoma are also at particularly high risk for t-MN (FIG. 1); up to 11% of patients treated with high-intensity chemotherapy develop t-MN[30]. This relatively higher risk likely stems, in part, from an inherited predisposition to cancer in one-half of these patients[31].

Changes in treatment protocols, including the decreased use of alkylating agents, have ushered in changes in t-MN incidence. Since 1975, the risk of t-MN in the United States has decreased for patients with ovarian cancer and multiple myeloma[21] (FIG. 1). By contrast, t-MN risk has increased for patients with non-Hodgkin lymphoma, particularly those who undergo allogeneic HSC transplantation (HSCT)[21]. In the 1990s, a new at-risk population emerged for patients with endometrial cancer, and since 2000, these at-risk populations have expanded to include patients with oesophageal, cervical and prostate cancer[21]. Another growing at-risk population is solid organ transplant recipients, who have an SIR of 4.6 for MDS and 2.7 for AML[22]. The expectation is that progress in developing targeted therapies with reduced nonspecific cytotoxicity will result in a decrease in t-MN incidence.

With respect to the latency period, patients diagnosed with a solid tumour have an increased risk of t-MN within 9 to 12 months after treatment. This risk peaks at 2 years and returns to a population baseline risk in 10–15 years[23]. The development of a t-MN after a primary haematological malignancy follows a broader

Standardized incidence ratio
(SIR). The ratio of the observed-to-expected number of cases based on demographic-specific incidence rates of acute myeloid leukaemia (AML) among the general population.

Fanconi anaemia
A bone marrow failure syndrome associated with an inherited mutation in one of at least 17 specific genes associated with the DNA damage response or DNA repair.

De novo AML
Acute myeloid leukaemia (AML) arising without a prior history of exposure to cytotoxic therapies or pre-existing myeloid neoplasm.

time course that peaks at 5 years and does not return to baseline even after 15 years; this risk pattern could be related to an underlying inherited predisposition to haematological malignancies, as discussed below.

Inherited risk factors

By convention, any myeloid neoplasia occurring after cytotoxic exposure is defined as therapy-related. The observation that some t-MN occur decades after cytotoxic therapy raises the question of whether they are a consequence of prior treatment. Instead, some may either represent second cancers arising in patients with a genetic predisposition or result from a stochastic (that is, random) second cancer. Some studies have associated t-MN with common inherited polymorphisms in genes encoding proteins involved in drug metabolism and DNA damage response pathways — for example, NAD(P)H quinone dehydrogenase 1 (*NQO1*), glutathione *S*-transferase P1 (*GSTP1*) and *RAD51* (reviewed in REF. 32). However, these are low-risk alleles. In the search for higher-penetrance inherited variants, researchers studied cancer survivors who developed t-MN and found that 16–21% of these survivors have a germline mutation associated with inherited cancer susceptibility genes[33–35]. Churpek and colleagues examined the frequency of germline mutations in 42 breast and ovarian cancer susceptibility genes in 47 breast cancer survivors who developed either a t-MN or therapy-related acute lymphoblastic leukaemia (ALL)[33]. Ten (21%) had germline mutations in either *BRCA1* or *BRCA2* ($n = 5$), *TP53* ($n = 3$), partner and localizer of BRCA2 (*PALB2*) ($n = 1$) or checkpoint kinase 2 (*CHEK2*) ($n = 1$). Other studies have corroborated germline mutations in *BRCA1*, *BRCA2*, *TP53* and Fanconi anaemia genes in patients with t-MN[34–39].

Many of the known t-MN predisposition genes encode components of the DNA damage response, which suggests a model whereby individuals with these germline mutations are particularly susceptible to cytotoxic chemotherapy and/or radiation because they have deficiencies in DNA repair, genomic instability, and/or insufficient cell cycle arrest and apoptosis. Supporting this hypothesis, BRCA1-deficient mice (*Brca1*$^{-/-}$) exhibit cytopenias, genomic instability and bone marrow failure, and a subset of these mice develop myeloid neoplasms[40]. Interestingly, breast cancers arising in patients with germline *BRCA1* or *BRCA2* mutations are associated with concurrent somatic *TP53* mutations, suggesting cooperativity of these genes[41,42]. We postulate that *TP53* dysfunction is integral to *BRCA1* or *BRCA2* mutation-associated tumours and potentially selects for the high frequency of *TP53* mutations in t-MN, a disease that is also characterized by defects in the DNA damage response[43].

An alternative but not mutually exclusive model is that genetically susceptible patients are at risk for the independent development of a second malignancy. Support for this model includes the extended latency period to t-MN development in patients with *BRCA1* or *BRCA2* mutations of 133 months[33]. In addition, among ten patients with germline mutations in any cancer susceptibility gene, five had either normal karyotypes or balanced recurring translocations in their leukaemia cells, which are cytogenetic findings more typical of *de novo* AML[24,33]. This supports the hypothesis that myeloid neoplasms arising in some genetically susceptible hosts are minimally influenced by the prior treatment and may represent independent second primary cancers.

Furthermore, germline mutations in genes other than those involved in the DNA damage pathways may interact with genotoxic cancer therapies to induce t-MN. For example, children with germline mutations of neurofibromin 1 (*NF1*), which encodes a negative regulator of RAS signalling, are at an elevated risk of developing t-MN[44]. Consistent with this idea, heterozygous *Nf1* mutant mice are strongly predisposed to radiation-induced myeloid neoplasms[45,46].

It is likely that expanding these studies to include larger gene panels or whole-genome sequencing in conjunction with the analysis of a larger cohort of patients will increase the number of known susceptibility genes and the frequencies of inherited risk alleles. Overall, emerging data emphasize the need to account for germline variants in the diagnosis and management of t-MN.

Table 1 | **Two major classes of therapy-related myeloid neoplasms**

	Alkylating agent class	Topoisomerase II inhibitor class
Cytogenetics	del(5q), –7/del(7q)*	t(11q23.3), t(21q22.1)
Frequency	~70% of t-MN patients	~30% of t-MN patients
Latency	5–7 years	2–3 years
Presentation	MDS	AML
Implicated drugs	• Alkylating agents: bendamustine, busulfan, carmustine, chlorambucil, cyclophosphamide, dacarbazine, lomustine, melphalan, mitomycin C, nitrogen mustard, procarbazine, thiotepa • Platinum-based agents: cisplatin, carboplatin • Antimetabolite agents: azathioprine, fludarabine	• Anthracyclines: daunorubicin, epirubicin, doxorubicin • Other topoisomerase II inhibitors: etoposide, teniposide, amsacrine, mitoxantrone

*Loss of the short arm of chromosome 17 containing the *TP53* gene due to del(17p), unbalanced rearrangement or –17 is observed in association with del(5q) in 40% of cases. AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; t-MN, therapy-related myeloid neoplasm.

Exposures

Chemotherapy. The risk of developing t-MN is likely to be determined by a complex interaction between the nature and dosage of the chemotherapeutic agent, the radiation intensity, genetic factors, the environment, comorbidities and the age of the patient. Although any cytotoxic agent can increase the risk of t-MN, alkylating agents and topoisomerase II inhibitors in particular are known to be causative (TABLE 1). Alkylating agents covalently modify the DNA, causing DNA crosslinking, DNA double-stranded breaks (DSBs), mutations and cytotoxicity. The cumulative dosage correlates with the incidence of t-MN, and some alkylating agents are more leukaemogenic than others[47,48]. Alkylating agents are

associated with longer latency to t-MN, a presentation of MDS that often progresses to AML, deletions of chromosome 5, loss or deletion of chromosome 7 and a poor prognosis[24]. Platinum-based chemotherapeutic agents have a weaker association with t-MN[49,50].

Topoisomerase II inhibitors induce DNA DSBs by preventing the religation of the DNA strands cleaved by topoisomerase II, which facilitates unwinding of the DNA during replication, and are correspondingly linked with balanced translocations[24]. Since myeloid leukaemias with balanced translocations, such as translocations involving *KMT2A* at 11q23.3, *RUNX1* at 21q22.1 or the promyelocytic leukaemia-retinoic acid receptor α (*PML–RARA*) fusion resulting from the t(15;17), have the fewest cooperating mutations[51], it is possible that the acquisition of multiple cooperating mutations is not required for leukaemogenesis and can explain why topoisomerase II inhibitor-associated t-MN has a shorter latency and presents as overt AML[24].

t-MN is also associated with prior exposure to the antimetabolites azathioprine and fludarabine[52,53]. However, it is difficult to parse out a definitive leukaemogenic role for other classes of drugs such as tubulin inhibitors, hydroxyurea, growth factors or other antimetabolites. This is partly because most patients receive combination therapies but may also be due to the weaker contributions of these other classes of drugs to t-MN. Among patients with ovarian cancer with germline *BRCA1* or *BRCA2* mutations, 0.8–2% developed t-MN after therapy with olaparib, a poly(ADP-ribose) polymerase 1 (PARP1) inhibitor, which suggests a unique susceptibility worthy of further study[54].

Radiation. Studies of atomic bomb survivors established radiation as a potent leukaemogen[55]. In cancer therapy, the incidence of myeloid neoplasia is proportional to the prior radiation dose[56], whereas the addition of radiation to a chemotherapy regimen increases the risk of t-MN in some, but not all, disease contexts[56]. For example, the risk of t-MN increases with combined modality treatment for lung and breast cancer, but this effect was not observed in patients with non-Hodgkin lymphoma, Hodgkin lymphoma or multiple myeloma[23]. The influence of radiation exposure was less apparent for these haematological malignancies, perhaps owing to the potency of the chemotherapy and/or the radiation field. It also remains possible that patients with primary haematological malignancies possess an underlying genetic predisposition that masks an additive effect of radiation.

The role of radiation therapy as a sole risk factor for t-MN development has been controversial. In t-MN that manifest following radiation monotherapy, 60% have abnormalities of chromosomes 5 and/or 7, which are similar to the cytogenetic abnormalities associated with alkylating agents; furthermore, t-MN due to radiation therapy present with a similar clinical and morphological profile to those associated with alkylating agent therapy[24]. The hope is that decreasing radiation fields of exposure and doses will be less leukaemogenic, as suggested by a more recent cohort of t-MN patients receiving radiation monotherapy who had clinical and cytogenetic features more similar to *de novo* myeloid neoplasms[57]. Furthermore, external beam radiotherapy or brachytherapy for prostate cancer may not be associated with an increased risk of MDS[58].

Somatic CGSs

The spectrum of genomic abnormalities in t-MN parallels that of *de novo* myeloid neoplasms, with the key distinction that t-MN is markedly skewed towards high-risk abnormalities[8]. Del(5q), –7/del(7q), complex karyotypes and *TP53* mutations all carry an adverse prognosis, and they are all profoundly over-represented in t-MN compared with *de novo* counterparts of t-MN[24] (FIG. 2). In contrast, favourable-risk abnormalities such as the t(8;21) or intermediate-risk normal karyotypes are considerably under-represented[24]. In this regard, t-MN parallels other high-risk myeloid neoplasms, namely, secondary AML arising from MDS[59] and *de novo* AML in elderly people[60].

A striking 70% of t-MN possess del(5q) or –7/del(7q) abnormalities as a result of deletions of the long arm of chromosome 5 or the loss or deletion of chromosome 7, respectively[24]. Long-standing efforts to identify a putative single tumour suppressor gene encoded within each of the commonly deleted regions were influenced initially by the dogma of Knudson's two-hit hypothesis and have been complicated more recently by the recognition of many potentially cooperating candidate genes within the deleted segments, suggesting the existence of CGSs within these loci (FIG. 3). With the exception of the gene that encodes polycomb repressive complex (PRC) component enhancer of zeste homologue 2 (*EZH2*)[61], 5q and 7q tumour suppressor genes have haploinsufficient phenotypes, and there is rarely a second inactivating mutation of the remaining allele[62]. Without a canonical 'second hit' to identify the tumour suppressor gene or genes, investigators mapped minimally deleted regions (MDRs) by examining large numbers of patients with myeloid neoplasms[62–65].

A CGS on the long arm of chromosome 5 (5q). There are two MDRs on chromosome 5q: 5q31.2 in patients with t-MN, *de novo* AML and high-risk MDS[63]; and 5q32 in those with 5q– syndrome, also known as MDS with isolated del(5q)[65]. Although earlier studies focused on genes in the MDRs, it is becoming clear that genes outside the MDR also contribute to disease. Moreover, the deletion in virtually all patients is large (~70 Mb), spanning 5q14–q33 and encompassing both MDRs, which makes it challenging to identify the involved genes[63,66]. Haploinsufficient genes implicated in del(5q) drive distinct disease characteristics, namely, anaemia (ribosomal protein S14 (*RPS14*) and adenomatous polyposis coli (*APC*)), megakaryocytic dysplasia (microRNA genes *mir-145* and *mir-146A*), HSC expansion (early growth response 1 (*EGR1*), *APC*, and casein kinase 1 α 1 (*CSNK1A1*)) and clonal dominance (*CSNK1A1*)[67–72]. Including *APC*, 5q14–q33 contains a total of 17 genes encoding components and regulators of WNT signalling, a pathway integral to haematopoiesis and leukaemogenesis[73].

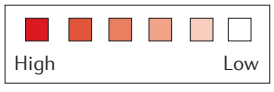
Brachytherapy
The use of radioactive sources implanted into the tumour tissue.

Knudson's two-hit hypothesis
A model stating that tumour suppressor genes are recessive and that inactivation of both alleles is required for a malignant phenotype.

5q– syndrome
A subset of MDS with an interstitial deletion of 5q as the sole cytogenetic abnormality (or with one additional abnormality). These patients present with macrocytic anaemia, megakaryocytic dysplasia and preserved or elevated platelet counts; additionally, they have a relatively favourable prognosis.

Cytogenetic abnormality	t-MN frequency (%)	*De novo* AML frequency (%)	Risk stratification	References
del(5q)*	42	5–16	Unfavorable	24, 162–164
–7/del(7q)*	49	4–14	Unfavorable	24, 162–164
Complex karyotype*	48	5–17	Unfavorable	24, 92, 163, 164
del(17p)/t(17p)/–17*,‡	20	4	Unfavorable	24, 162–164
+8	3	10–13	Unfavorable	24, 162–164
Normal karyotype	8	41–48	Intermediate	24, 162–164
t(11q23.3)	3	3–4	Intermediate	24, 162–164
inv(16), t(16;16)	2	1–7	Favorable	24, 162–164
t(15;17)	2	3–13	Favorable	24, 162–164
t(21q22.1)	3	2–7	Favorable	24, 162–164

Gene mutation	t-MN frequency (%)	*De novo* AML frequency (across karyotypes) (%)	References
ABC family	14	4	6
ASXL1	3–17	3–11	6, 8, 51, 153, 165
BCOR	1–3	1	6, 8, 51
CBL	2–4	1	6, 8, 51
CEBPA	0–5	6–9	8, 51, 154, 182
CUX1	2	0–1	8, 51, 79
DNMT3A	8–27	14–25	6, 8, 51, 153, 183
ETV6	1–3	1–2	6, 8, 51, 166
EZH2	3–4	2	6, 8, 51, 153, 184
FLT3	8–16	24–28	6, 8, 51, 154, 167
IDH1	3–5	8–10	6, 51, 153, 155, 168
IDH2	0–5	9–10	6, 51, 153, 155, 168
JAK2	0–1	1–2	8, 51, 156, 169
KIT	0–3	4–6	6, 8, 51, 154, 182
KMT2A (ITD)	3	5	157, 170
KRAS	11	2–4	8, 51, 182
NF1	2–4	2	6, 8, 51
NPM1	4–16	27–35	6, 8, 51, 158, 171
NRAS	10–13	8–10	8, 51, 172
PHF6	1–3	3	6, 8, 51, 173
PTPN11	3–9	5	6, 8, 159, 51
RAD21	4	1–2	8, 51, 174
RUNX1	11–16	6–13	6, 8, 51, 160, 175, 176
SETBP1	3	0–1	8, 51, 161, 177
SF3B1	0–3	1–5	8, 51, 153, 178
SMC1A	3	1–4	8, 51, 174
SMC3	2	1–4	8, 51, 174
SRSF2	8–11	1	8, 51, 153
STAG2	5–6	1–3	6, 8, 51, 174
TET2	6–14	8–27	6, 8, 51, 153, 179, 181, 182
TP53	23-37	2–12	6, 8, 51, 91, 92, 153, 182
U2AF1	5–8	4	6, 8, 51
WT1	2–3	6–8	6, 8, 51, 182
ZRSR2	1	0	8, 51



A CGS describes a disease involving multiple dose-sensitive genes within a genomic interval[74] in which an *en bloc* segmental deletion is more pathogenic than inactivation of any single gene. For example, *Trp53* reduction combined with haploinsufficiency of the 5q-encoded gene *Egr1* does not lead to overt leukaemia in mice, whereas *Trp53* reduction in combination with haploinsufficiency of the 5q-encoded genes *Apc* and *Egr1* does[9]. CGSs in cancer can occur with other recurring deletions in myeloid neoplasms, that is, del(7q) (see below), del(20q) and deletions encompassing *TP53* at 17p13.1 (REF. 75), *RB1* at 13q[76] and *MYC* at 8q24.21 (REF. 77), to name a few. Additionally, these CGSs may be relevant to the large segmental deletions characteristic of common epithelial tumours, such as breast, colon and lung carcinomas[78].

A CGS on 7q. Chromosome arm 7q contains three MDRs (FIG. 3). At 7q22, cut like homeobox 1 (*CUX1*) is a haploinsufficient tumour suppressor gene that is recurrently mutated in haematopoietic malignancies and solid tumours[10,79]. The genetic evidence for *CUX1* acting as a tumour suppressor gene is strong, as heterozygous *CUX1*-inactivating mutations are independently associated with a poor prognosis[79]. Heterozygous deletion of a 2 Mb syntenic interval near *Cux1* led to features of MDS in mice, demonstrating that 7q22 is also a CGS region[11]. The 7q34 locus contains LUC7 like 2, pre-mRNA splicing factor (*LUC7L2*), which encodes a spliceosomal

Figure 2 | **Recurrent mutations and cytogenetic abnormalities in therapy-related myeloid neoplasms.** The frequencies (%) of cytogenetic abnormalities and somatic gene mutations are shown for therapy-related myeloid neoplasms (t-MN) and *de novo* acute myeloid leukaemia (AML). The colour intensity of the heatmap reflects the frequency, and ranges from low (white) to high (bright red) frequency. The data are from the following references: t-MN[6,8,24,92,153–161] and *de novo* AML[79,162–183]. ABC, ATP-binding cassette; *ASXL1*, additional sex combs like 1; *BCOR*, BCL6 co-repressor; *CBL*, encoding a ubiquitin ligase; *CEBPA*, CCAAT/enhancer binding protein α; *CUX1*, cut like homeobox 1; *DNMT3A*, DNA methyltransferase 3A; *ETV6*, ETS variant 6; *EZH2*, enhancer of zeste homologue 2; *FLT3*, fms related tyrosine kinase 3; *IDH1*, isocitrate dehydrogenase 1; ITD, internal tandem duplication; *JAK2*, Janus kinase 2; *KMT2A*, histone-lysine *N*-methyltransferase 2A; *NF1*, neurofibromin 1; *NPM1*, nucleophosmin 1; *PHF6*, PHD finger protein 6; *PTPN11*, protein tyrosine phosphatase, non-receptor type 11; *RUNX1*, runt related transcription factor 1; *SETBP1*, SET binding protein 1; *SF3B1*, splicing factor 3b subunit 1; *SMC*, structural maintenance of chromosomes; *SRSF2*, serine and arginine rich splicing factor 2; *STAG2*, stromal antigen 2; *TET2*, tet methylcytosine dioxygenase 2; *U2AF1*, U2 small nuclear RNA auxiliary factor 1; *WT1*, Wilms tumour 1; *ZRSR2*, zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2. *Denotes that del(5q), –7/del(7q) and a complex karyotype can co-occur in the same patient; thus, these frequencies partially overlap. ‡Of note, 17p deletions are often cryptic and therefore are under-reported by conventional cytogenetic analysis. Thus, the numbers shown may represent the lower range of the true frequency.

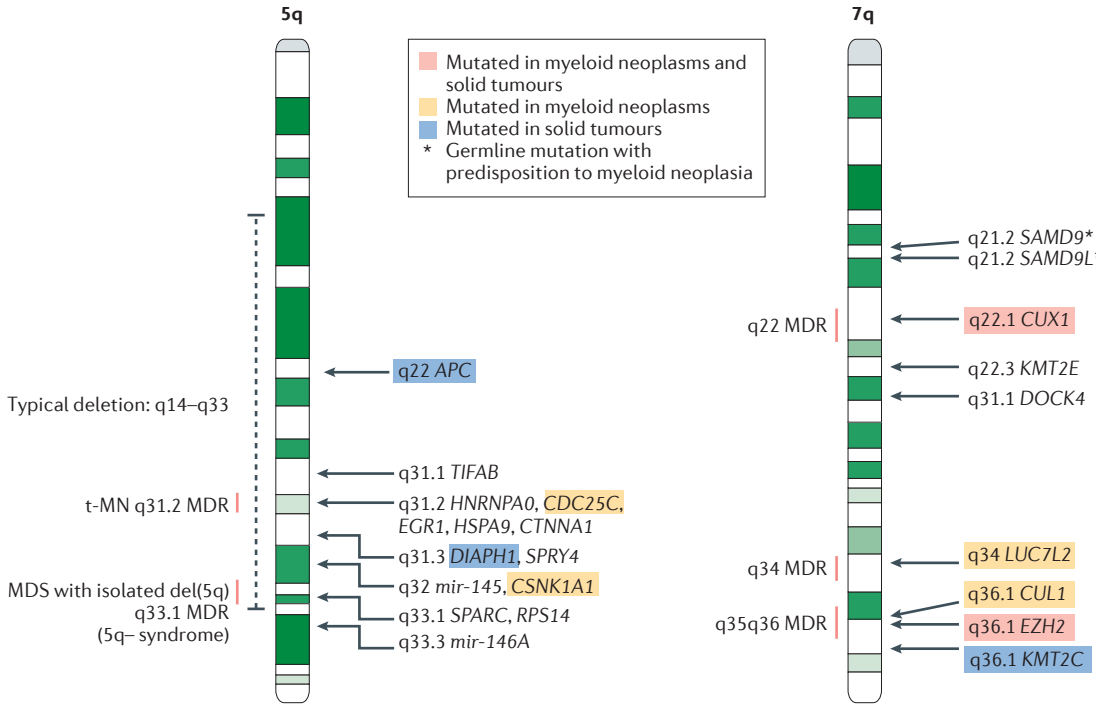


Figure 3 | **Contiguous gene syndrome regions on chromosomes 5 and 7.** Minimally deleted regions (MDRs) on the long arms of chromosomes 5 (REFS 63,65) and 7 (REFS 64,184,185) are shown. In myeloid neoplasms, there is evidence for a role for haploinsufficiency of the following genes on 5q: adenomatous polyposis coli (*APC*)[67], TRAF-interacting protein with forkhead-associated domain B (*TIFAB*)[186] and early growth response 1 (*EGR1*)[187]. Heterogeneous nuclear ribonucleoprotein A0 (*HNRNPA0*)[188], heat shock protein family A (Hsp70) member 9 (*HSPA9*)[189], diaphanous related formin 1 (*DIAPH1*)[190], and sprouty 4 (*SPRY4*)[191] have been implicated based on mouse models, and mutations of cell division cycle 25C (*CDC25C*)[192] have been identified in familial platelet disorder (FPD) progressing to acute myeloid leukaemia (AML). *CTNNA1* (encoding α-catenin)[193] has been implicated in some, but not all, studies. The microRNA genes *mir-145* (REF. 69) and *mir-146A*[69], casein kinase 1 α 1 (*CSNK1A1*)[71], secreted protein acidic and cysteine-rich (*SPARC*)[194], and ribosomal protein S14 (*RPS14*)[68] have been implicated in the pathogenesis of myelodysplastic syndrome (MDS) with an isolated del(5q). On 7q, there is evidence for a role in myeloid neoplasia for these genes: sterile alpha motif domain containing protein 9 (*SAMD9*)[85], *SAMD9L*[82,83], cut like homeobox 1 (*CUX1*)[10], histone-lysine *N*-methyltransferase 2E (*KMT2E*)[195–197], dedicator of cytokinesis 4 (*DOCK4*)[198], LUC7 like 2, pre-mRNA splicing factor (*LUC7L2*)[80], cullin 1 (*CUL1*)[80], enhancer of zeste homologue 2 (*EZH2*)[199] and *KMT2C* (also known as MLL3)[81]. Blue boxes indicate genes significantly and recurrently mutated in pan-cancer analysis[200]. Yellow boxes indicate genes recurrently mutated in myeloid neoplasms. Pink boxes indicate genes recurrently mutated in both myeloid and pan-cancer. Note that *KMT2C* mutations may be overestimated due to a pseudogene causing false-positive mutations[201]. t-MN, therapy-related myeloid neoplasms. *Genes with inherited mutations that are associated with a predisposition to myeloid dysplasia are indicated.

Ataxia-pancytopenia syndrome
Also known as myelocerebellar disorder; associated with ataxia, bone marrow failure and a predisposition to myeloid leukaemia with monosomy 7.

Revertant mosaicism
When a disease-causing mutation is spontaneously somatically corrected for and the corrected cell clonally expands.

Shwachman–Diamond syndrome
An inherited disorder associated with skeletal abnormalities, exocrine pancreatic insufficiency and bone marrow failure that may progress to myeloid leukaemia with chromosome 7 abnormalities.

protein that is mutated in myeloid malignancies[80]. A third MDR at 7q35–q36 contains cullin 1 (*CUL1*) and *EZH2*, both of which have been reported to be somatically mutated in myeloid neoplasms[61,80]. This distal CGS segment also contains the haploinsufficient tumour suppressor gene *KMT2C* (also known as *MLL3*)[81].

Monosomy 7 or large 7q deletions will include sterile alpha motif domain containing protein 9 like (*SAMD9L*) at 7q21.2; this gene encodes a facilitator of endosome fusion[82]. Inherited *SAMD9L* mutations cause ataxia-pancytopenia syndrome and a predisposition for AML with monosomy 7 (REF. 83). These patients have been observed to develop mosaic 7q loss of heterozygosity in their peripheral blood cells, leading to loss of the mutant *SAMD9L* allele[83]. This is intriguing because revertant mosaicism of chromosome 7 has been reported in Shwachman–Diamond syndrome[84], a syndrome linked to *SBDS* gene mutations at 7q11.21 and a predisposition to AML with –7/del(7q). *SAMD9* encodes a *SAMD9L* paralogue and is located on chromosome band 7q21.2. Adaptation by aneuploidy is also reported in patients with *SAMD9* mutations with a predisposition to MDS with monosomy 7 (REF. 85). The mutated SAMD9 and SAMD9L proteins in these patients were found to restrict cell growth more than their wild-type counterparts. Thus, cells from individuals exhibiting a loss of heterozygosity of chromosome 7, which carries the mutant allele may have undergone adaptation to the growth restriction. It is tempting to speculate that monosomy 7 in some diseases with a predisposition to AML with –7/del(7q) is a consequence of the pressure for revertant mosaicism.

Somatic gene alterations

NGS of genomes has yielded surprising results and transformed our understanding of the aetiology and pathogenesis of t-MN. The most comprehensive

Transition-type mutations
A DNA mutation that changes a purine to a different purine nucleotide or a pyrimidine to a different pyrimidine.

Aplastic anaemia
A disorder characterized by pancytopenia that confers risk of transformation to myelodysplastic syndrome (MDS) or acute myeloid leukaemia (AML) and occurs as a result of either germline mutations or acquired immune destruction of haematopoietic precursors.

study published to date analysed the whole genomes of 22 patients with t-MN as well as 149 AML and/or MDS-related genes in an additional 89 patients[6]. The expectation was that because all 22 patients had been exposed to chemotherapy and/or radiation therapy for their primary diseases, the burden of somatic mutations would be greater than that found in *de novo* AML due to the ensuing DNA damage[6]. Surprisingly, the numbers of single nucleotide variants (SNVs) and small insertions and deletions (indels) as well as copy number alterations (CNAs) were similar in t-MN and *de novo* AML, with each t-MN genome carrying ten SNVs or indels in coding regions[6]. Overall, this is one of the lowest mutational burdens across human cancers[86]. We also noted a low mutational load in the exome and RNA-sequencing analysis of ten t-MN cases[87]. Similarly, a study of the mutations in 53 genes in 70 t-MN patients found that the mutation rate was comparable to that of *de novo* AML and/or MDS[7]. These findings are contrary to the historical premise that t-MN largely arises owing to chemotherapy and/or radiation-induced mutations.

Furthermore, if chemotherapy drives somatic mutational changes in t-MN, it would be expected to leave a signature in the mutation spectra. Alkylating agents preferentially induce transition-type mutations[88], and this signature is apparent in glioblastomas in patients who were pretreated with alkylating agents before surgical resection[86]. Even though at least half of the t-MN patients had a history of exposure to an alkylating agent, the frequency of transition-type mutations was similar in t-MN patients compared with *de novo* AML[6]. Thus, the lack of a chemotherapy-induced mutational signature combined with the surprisingly low mutational load suggests that treatment-induced mutation is not the only or major driver of t-MN. In addition, the low number of DNA base-level mutations raises the question of whether large-scale genomic alterations such as chromosomal deletions or balanced translocations are the critical defects in the pathobiology of t-MN. Nonetheless, it cannot be ruled out from these studies that chemotherapy induces mutations in some cases. For example, there is a clear relationship between the mechanism of action of topoisomerase II inhibitors and the induction of balanced translocations[24,89] (FIG. 4). In contrast, alkylating agents can cause DNA DSBs, which can lead to a chromosomal deletion[90]. On average, seven somatic CNAs have been identified in t-MN patients who received prior alkylating agent therapy (James Downing and Joy Nakitandwe, unpublished data), whereas CNAs are infrequent in patients treated with a topoisomerase II inhibitor [6]. CNAs are skewed 2:1 in favour of loss versus gain of chromosomal material, with patients possessing a del(5q) abnormality showing a 2-fold increase over the average; this frequency is consistent with the association with a complex karyotype.

In addition to a comparable number of mutations and type of mutation, a growing number of studies report that the mutated genes and altered pathways — that is, genes encoding components of the spliceosome, chromatin modifiers, transcription factors and receptor tyrosine kinase signalling proteins — are completely overlapping in t-MN and *de novo* AML (FIG. 2). Ebert and colleagues[8] report that the overall spectrum of mutated genes in t-MN is indistinct from that in *de novo* AML. t-MN somatic mutations were more akin to *de novo* AML with the same cytogenetic changes than to t-MNs with other cytogenetic changes. Thus, the emerging pattern indicates that when analyses control for karyotype, *de novo* AML and t-MN are indistinguishable at the genetic level. In other words, t-MN have a significantly higher frequency of high-risk karyotypes (for example, complex karyotypes and abnormalities of chromosomes 5 and/or 7 are seen in ~70% versus 20% in cases of *de novo* AML), but t-MNs with high-risk karyotypes are indistinguishable from the mutational, clinical and morphological profile of *de novo* AML with comparable high-risk karyotypes.

In t-MN, there are two broad genetic pathways that account for the majority of cases. *TP53* mutations define the first and occur in up to 37% of t-MN[91]. t-MN and *de novo* AML with *TP53* mutations are associated with del(5q) abnormalities in ~80% of cases as well as complex karyotypes, resistance to therapy and poor overall survival[92]. The –7/del(7q) abnormality defines the second major subgroup of t-MN, occurs in 49% of patients[24] and is associated with mutations that activate the RAS pathway in both *de novo* AML and t-MN[87,93]. Although this subgroup also has a poor prognosis, the development of RAS pathway inhibitors provides some therapeutic promise[94]. There is partial overlap between the *TP53*-mutated subgroup and the –7/del(7q) subgroup, as the –7/del(7q) abnormality may also occur in *TP53*-mutated cases, typically in the context of a del(5q) abnormality and a complex karyotype[24,87]. The remaining t-MN cases that do not fall into these two ontogenies are akin to *de novo* counterparts (defined by balanced translocations or a normal karyotype with nucleophosmin 1 (*NPM1*) and/or fms related tyrosine kinase 3 (*FLT3*) mutations, among others)[8].

To date, there is no clear sequence of genetic events that produce t-MN, and multiple scenarios likely exist. *TP53* mutations can be the initiating lesion in some t-MN[6,95]. In MDS with a del(5q), this deletion is the initiating defect in up to 73% of cases, and *TP53* mutations are acquired as a secondary cooperating mutation associated with a high risk of progression to AML[96,97]. Monosomy 7 occurs in the absence of recurrent gene mutations in most MDS cases associated with aplastic anaemia[98]. Future studies to illuminate the sequence of events and how the order of mutations alters the disease phenotype will be informative.

The role of clonal haematopoiesis

In 1993, Cachia *et al.*[99] reported a higher frequency of clonal haematopoiesis in patients who had received cytotoxic therapy for lymphoma and presciently suggested that these patients should be followed prospectively for t-MN development. More recent reports suggest that up to 15% of patients with breast and/or ovarian cancer who were treated with one round of chemotherapy and 27% of patients who received two rounds of chemotherapy developed clonal haematopoiesis[100–102]. Clonal haematopoiesis of indeterminate potential (CHIP) is the presence of a clonal population of haematopoietic cells with

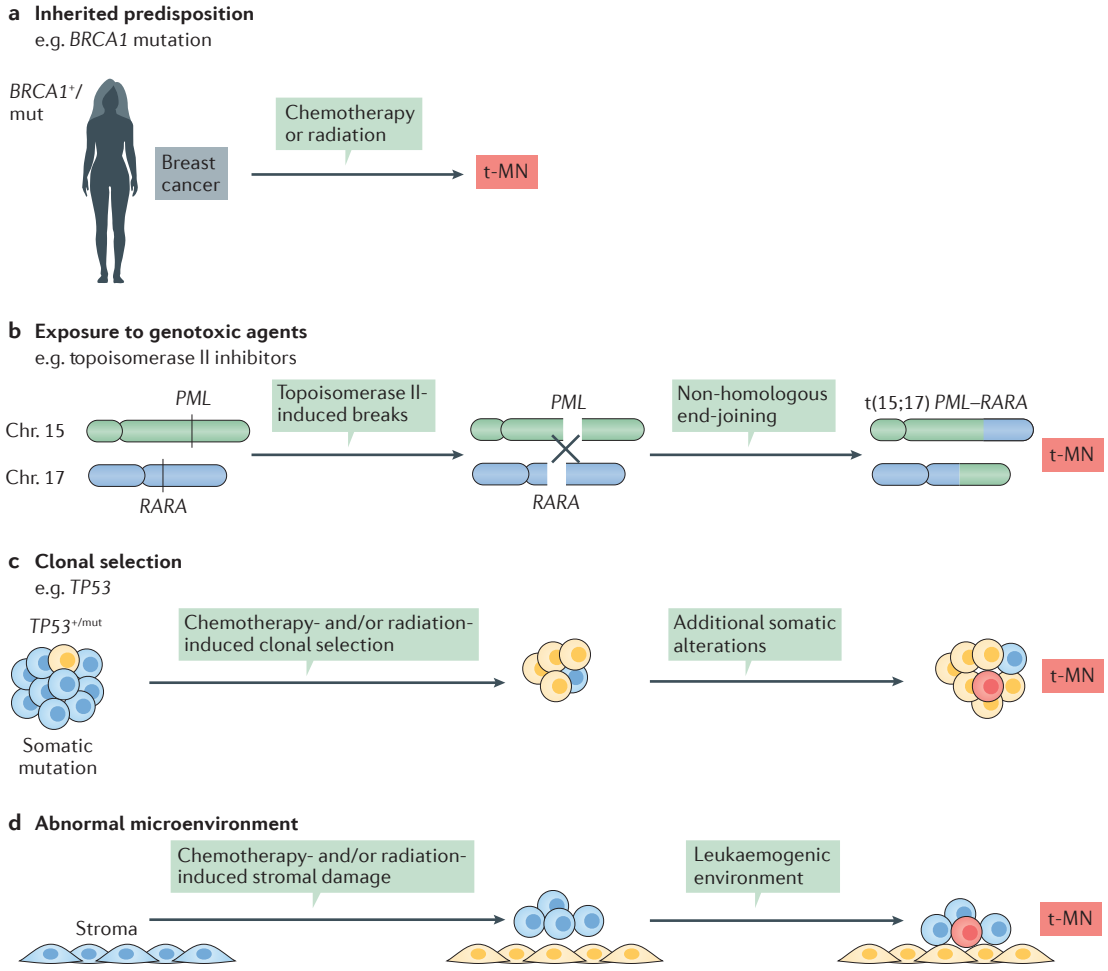


Figure 4 | **Both intrinsic and extrinsic factors contribute to the development of therapy-related myeloid neoplasms.** There are four main drivers for therapy-related myeloid neoplasm (t-MN) development. These pathways are not mutually exclusive, and one or multiple pathways may contribute to t-MN development in any individual patient. **a** | Patients with an inherited mutation in a gene encoding a component of the DNA damage response and DNA repair pathways have an increased susceptibility to the development of multiple, independent malignancies. In addition, these patients may have increased susceptibility to chemotherapy and/or radiation-induced mutations due to an impaired DNA damage response or DNA repair pathway. **b** | The role for therapy as a mutagen is illustrated. In the prevailing model of t-MN, chemotherapy and/or radiation induces DNA damage that leads to transformation. An example of this is topoisomerase II inhibitor-induced translocations. **c** | The role for therapy as a driver of clonal selection is shown. In patients with a pre-existing somatic mutation and clonal haematopoiesis, that is, clonal haematopoiesis of indeterminate potential (CHIP), chemotherapy and/or radiation gives these clones a competitive advantage over normal haematopoietic stem cells (HSCs). The consequences of the somatic mutation — for example, genetic instability associated with mutant *TP53* — combined with regenerative haematopoiesis facilitates the acquisition of subsequent mutations and t-MN development. **d** | Chemotherapy and/or radiation also induce damage to the bone marrow stromal niche. Alternatively, the bone marrow microenvironment may be altered or damaged as a result of normal processes, for example, ageing. Altered niche function, such as a pro-inflammatory response, may promote clonal selection and/or produce factors that favour leukaemia-initiating cells. Chr., chromosome; mut, mutant; *PML*, promyelocytic leukaemia; *RARA*, retinoic acid receptor α.

a somatic mutation in a gene associated with haematological malignancies in the absence of morphological evidence of disease. The fundamental role of CHIP in the pathogenesis of t-MN is now becoming apparent[103].

Almost 1% of healthy individuals harbour clonal, somatic, numerical or structural chromosomal changes in the blood[104–107]. The frequency increases to 2–3% in individuals over age 60 (REFS 106,107), and some clones expand over time[105,108]. The types of abnormality — such as del(20q), del(13q), del(11q), trisomy 8 or, less commonly, del(5q) or del(7q) — even include those found in haematological malignancies[104,105,107]. Not surprisingly, clonal mosaicism for structural rearrangements carries a tenfold increased risk of a subsequent haematological malignancy[107,109].

NGS studies of peripheral blood lymphocytes have identified that gene-level somatic mutations and evidence of CHIP increase in incidence with age and have an association with the subsequent development of a haematological cancer[110–113]. Ultrasensitive sequencing

revealed CHIP in 19/20 (95%) healthy individuals 50–60 years of age[114]. The genes mutated in CHIP are also recurrently mutated in myeloid malignancies, for example, DNA methyltransferase 3A (*DNMT3A*), additional sex combs like 1 (*ASXL1*), tet methylcytosine dioxygenase 2 (*TET2*) and *TP53*.

How might pre-existing somatic mutations and CHIP relate to t-MN? Over 20 genes provide an engraftment advantage to HSCs when inactivated[115], including *DNMT3A* in humans and *Tet2* in mice[116–118]. We propose a model whereby mutated HSC clones with a 'fitness' advantage will preferentially survive during chemotherapy and/or radiation and then repopulate the haematopoietic compartment. Those same mutations may also cause abnormal differentiation and genomic instability, which is a recipe for dysfunctional haematopoiesis and, ultimately, transformation. The relative risk of individual mutant genes and structural rearrangements for subsequent t-MN is an active area of investigation.

Recent reports have established direct links between pre-existing somatic mutations and t-MN. Wong and colleagues[6] identified somatic *TP53* mutations in the blood of healthy individuals. They and others demonstrated that *TP53* mutations are detectable in the blood of some patients with t-MN before their exposure to chemotherapy for the primary malignant disease[6,95]. In addition, HSCs from *Trp53*$^{+/-}$mice have a clonal advantage after chemotherapy and/or radiation[6,119].

Expanding on this result, a retrospective study of t-MN patients identified CHIP in 10/14 (71%) patients at the time of the primary malignancy before they received treatment[120]. Two additional retrospective studies reported CHIP in 62–66% of t-MN patients at the time of the primary malignancy, although most of these patients had already received some type of treatment[121,122]. The positive predictive value of CHIP for the development of t-MN was 27–35%, and the negative predictive value was 89–98%, thereby providing the first potential biomarker for t-MN[120,121]. There is some indication that certain mutated CHIP genes are more predictive of subsequent t-MN. However, the number of patients studied is currently too small to achieve a consensus on which genes are more informative. Higher clonal frequency and more than one gene mutation also indicated a greater risk of t-MN[120,122]. Whether the presence of multiple mutations occurs within the same clone and whether this is clinically meaningful remains unknown. In most cases, the mutation present in the CHIP clone was identified in the t-MN and likely drove the pathogenesis of the myeloid neoplasm. However, in a few patients, the CHIP mutation was not present in the t-MN, suggesting that, in these cases, CHIP may indicate diseased bone marrow and/or genetic instability. In light of the finding suggesting that most individuals over the age of 50 have CHIP[114], it is imperative to define the features of CHIP that are clinically relevant and predictive of the development of subsequent myeloid neoplasia.

In summary, there is strong evidence that chemotherapy and/or radiation creates an environment that selects for pre-existing mutant clones at the expense of normal, healthy HSCs (FIG. 5). This also partially explains why t-MN are inherently resistant to treatment: the mutant leukaemia-initiating clones have already undergone one or more rounds of selection for increased 'fitness' in response to chemotherapy and/or radiation.

It could be argued that clonal selection is the origin of other high-risk myeloid neoplasms with inherent chemoresistance (BOX 1). AML in elderly people has high-risk clinical and genetic features often indistinguishable from t-MN[60]. In this instance, HSC oligoclonality associated with normal ageing and increased potential for chemical and/or environmental exposures during the lifetime of an individual may drive HSC selection. Clonal competition may arise during regenerative haematopoiesis driven by normal processes, such as ageing, inflammation, immune destruction, viral infections, defects in the bone marrow niche and/or dysfunctional haematopoiesis, as found in aplastic anaemia and MDS. Indeed, CHIP occurs in 47–73% of patients with aplastic anaemia, a patient population with a known risk of developing either AML or MDS with high-risk cytogenetic features[123–125]. Likewise, secondary AML arising from MDS has high-risk features akin to t-MN[59].

The role of the bone marrow niche

Recent studies highlight the role of the complex bidirectional crosstalk between HSCs and the bone marrow niche in normal haematopoiesis as well as in the pathogenesis of myeloid diseases[12–15]. Multiple cell types contribute to the niche, including various mesenchymal stromal cell (MSC) populations and progeny cells derived from MSCs, such as osteoblasts[126]. Emerging data suggest that niche alterations play a role in the pathogenesis of myeloid neoplasms[12–15], although these results are not fully understood. Functional defects of the MDS and/or AML niche have been noted, such as a decrease in the production of HSC-supportive factors[127,128], and may account in part for the poor results of HSC transplantation in patients with t-MN and high-risk MDS and AML. Medyouf *et al.*[128] showed that MSCs from MDS patients have a perturbed differentiation programme and are essential for the propagation of MDS HSCs ($CD34^+$, $CD38^-$) in mouse xenograft models. Healthy MSCs adopted MDS MSC-like molecular features when exposed to MDS HSCs via niche reprogramming[128]. Conversely, other studies in mice revealed that genetic changes in MSCs (CREB-binding protein (*Crebbp*)$^{+/-}$, *Rara*$^{-/-}$ or *Apc*$^{del/+}$) or osteoblasts (*Dicer1*$^{-/-}$) led to the development of MDS and AML[13,15,129,130].

In the past decade, alterations in several cellular pathways in the bone marrow niche have been implicated in the aetiology of myeloid diseases. Canonical WNT signalling plays a role in the regulation of haematopoiesis, the aberrant renewal of leukaemia stem cells and the maintenance of bone marrow niche function[131,132]. Gene expression profiling of haematopoietic cells has identified WNT pathway activation in MDS, AML and t-MN with del(5q)[73,133,134]. Deregulation of WNT–β-catenin signalling in cancer has been correlated with genomic instability, raising the possibility that this pathway contributes to genomic instability and complex karyotypes characteristic of this subtype

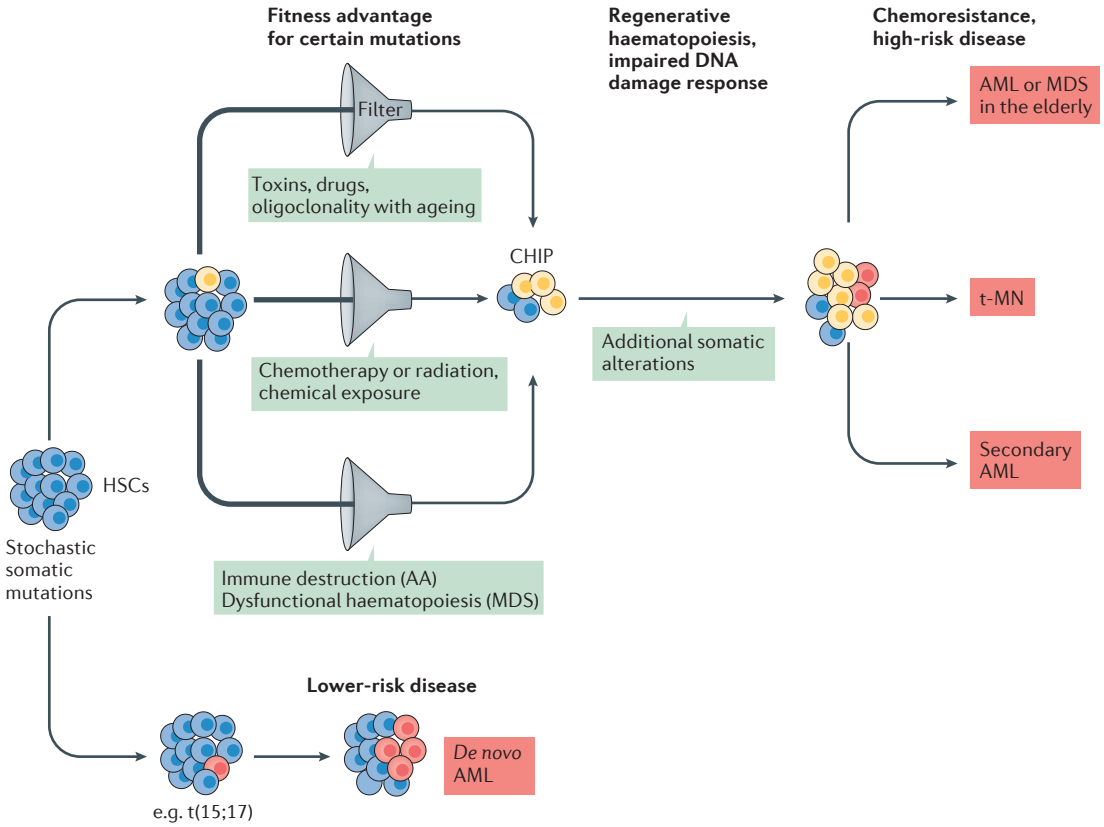


Figure 5 | **Model for the role of clonal selection in the aetiology of high-risk myeloid neoplasms.** Stochastic mutations occur in haematopoietic stem cells (HSCs) over time. Certain mutated genes provide a 'fitness' advantage in the context of various competitive conditions. Competition 'filters' mutant HSCs at the expense of healthy HSCs, resulting in clonal haematopoiesis of indeterminate potential (CHIP). Over time, mutant clones can acquire additional mutations due to mutations in DNA damage response genes and/or increased proliferation in the context of regenerative haematopoiesis. In haematopoiesis, competition arises in a number of different contexts. Loss of HSC diversity with age and the cumulative lifetime exposure to toxins and drugs, among other factors, may select for mutant clones that ultimately give rise to myeloid neoplasms in elderly people. Chemotherapy and radiation exposure promotes therapy-related myeloid neoplasms (t-MN). Inflammation, immune destruction and dysfunctional haematopoiesis (including bone marrow niche-based effects) can give rise to secondary myeloid neoplasms arising from aplastic anaemia (AA) or myelodysplastic syndrome (MDS). Regardless of the selective pressure, the competitive filter increases the likelihood that the malignant clones will have inherent resistance to therapy. In contrast, a stochastic mutation that gives rise to AML without an antecedent clonal selection is more likely to be a lower-risk disease.

of t-MN. In parallel, activation of β-catenin, the nuclear mediator of WNT signalling, was observed in osteoblastic cells of the bone marrow niche with a resultant increase in Notch signalling in HSCs in ~40% of MDS and AML patients studied, most (80%) of whom had del(5q) and/or –7/del(7q) abnormalities[135].

In elegant work using mouse models of MDS, Zambetti *et al.*[136] established mechanistic links between inflammation and cancer. Specifically, they demonstrated that overexpression of the damage-associated molecular pattern (DAMP) genes S100A8 and S100A9 in the mesenchymal niche cells drives genotoxic stress in HSCs via activation of the p53–S100A8/9–Toll-like receptor 4 (TLR4) signalling axis. Moreover, transcriptional activation of this signalling axis in the bone marrow niche predicted progression to AML and survival in patients with MDS, providing a rationale for therapeutic targeting of the bone marrow niche-based inflammatory signalling in some myeloid disorders[136].

What then is the role of cytotoxic therapy in this process? The answer is likely to be complex, as cytotoxic therapy has been shown to exert a number of effects on the bone marrow microenvironment, including a pro-inflammatory response and the consequent release of inflammatory cytokines, for example, tumour necrosis factor (TNF), transforming growth factor β (TGFβ), and interleukin-6 (IL-6); the release of reactive oxygen species (ROS) by MSCs with resultant genotoxic damage to HSCs; injury to sympathetic nerves and remodelling of the bone marrow niche. In a mouse model of t-MN, based on haploinsufficiency of two del(5q) genes, *Egr1* and *Apc*, together with *Trp53* knockdown, Stoddart *et al.*[130] observed a high frequency of myeloid diseases following concurrent treatment of both haematopoietic cells and the bone marrow stroma with an alkylating agent, but not after treatment of either alone. These observations reinforce the role of the intricate bidirectional interplay between mutant haematopoietic stem and progenitor

Performance status
Measure of physical functioning of the patient to help predict prognosis.

cells and MSCs in myeloid diseases (Stoddart, Le Beau, *et al.*, unpublished results). Link and Walter[137] proposed that HSC-extrinsic factors such as cytotoxic therapy, toxins, infection and/or inflammation, or autoimmunity — processes known to impact the bone marrow niche — may transiently reduce the size of the HSC pool and contribute to clonal selection of mutant HSCs and subsequent disease development. There is strong evidence that cytotoxic therapy creates an environment that selects for pre-existing mutant clones at the expense of normal, healthy HSCs. In this context, DNA damage-induced competition led to a selective clonal advantage of HSCs and haematopoietic progenitor cells with reduced p53 function (and possible clonal expansion of emerging cancer cells) in mouse bone marrow chimaeras — reminiscent of the CHIP phenotype — via growth arrest and senescence-related gene expression in cells with higher p53 activity[119]. Additionally, this competition possibly induced the expression of genes that reduce apoptosis. Interestingly, the extent of cell competition as a result of reduced p53 levels and the corresponding selective advantage was greater in individuals with a smaller proportion of p53-deficient cells in the initial haematopoietic stem or progenitor cell population. This was possibly due to the phenomenon of 'frequency-dependent selection', whereby rare p53-low cells largely compete with cells with wild-type levels of p53 rather than with each other. Moreover, genes encoding proteins involved in interactions between haematopoietic cells and cells of the microenvironment (for example, adhesion and migration) were differentially expressed in HSCs with a clonal selective advantage, implicating a role for p53 levels in mediating a gene expression profile in HSCs that can alter interactions with the stroma. This 'clonal selection model' is a paradigm shift and will impact our understanding of the biology of t-MN, the development of treatments to prevent t-MN and the screening methods to use for t-MN.

In summary, studies in mouse models and in patients have indicated that treatment-induced mutations in HSCs are not the only driver of t-MN and additional factors, such as bone marrow niche-induced changes, also influence disease pathobiology. Specifically, bone marrow stromal changes and the formation of a malignant niche are not merely a consequence of the malignant process but contribute directly to the pathogenesis of the disease.

Box 1 | **Tenets of the clonal selection model of cancer**

1. Somatic mutations occur in tissue stem cells
2. The prevalence of mutations accumulates with age
3. Some mutated genes provide a 'fitness' advantage
4. Competitive conditions select for mutated clones
   a. In the haematopoietic compartment, competition may arise from diverse causes: regenerative haematopoiesis, chemotherapy, radiation, chemical exposure, infections, inflammation, immune destruction (aplastic anaemia, paroxysmal nocturnal haemoglobinuria), oligoclonal haematopoietic stem cells (HSCs) associated with ageing, defects in the niche and dysfunctional haematopoiesis (myelodysplastic syndrome (MDS), aplastic anaemia)
   b. In non-haematopoietic tissues, competition may arise from similar causes: regenerative growth, chemotherapy, radiation, chemical exposure, infections, inflammation, immune destruction, oligoclonal stem cells associated with ageing and defects in the niche
5. Clones acquire additional mutations due to increased cell division and/or inactivation of the DNA damage response pathway
6. The malignant clones have inherent resistance to therapy because they have already undergone one or more rounds of selection for increased 'fitness' in response to competitive pressures

Prognosis

The outcome for t-MN patients is poor, with a 5-year survival of 10%[20]. In the largest study carried out by a single institution, which recruited 303 t-MN patients between 1972 and 2001, the median survival was 8.0 months[24]. Two decades later, a modest improvement in survival (14.6 months) was reported in a multicentre study of 277 patients recruited between 1999 and 2013 (REF. 20). Older age at t-MN diagnosis, unfavourable karyotype (FIG. 2), low haemoglobin and low platelet counts portend a worse outcome[20].

Compared with patients with *de novo* AML, t-MN patients are more likely to have high-risk karyotypes, comorbidities and poor performance status[138]. In a study that adjusted for these confounding factors, it was found that among patients 60 years or older with a high-risk karyotype and who were undergoing intensive therapy, t-MN patients had a similar outcome as patients with *de novo* AML[138]. This observation suggests that these diseases are biologically similar in older patients. For younger patients and patients with favourable-risk karyotypes, t-MN is an independent adverse prognostic indicator[138–140].

HSCT significantly improves patient survival to 58.8 months and is the only potentially curative treatment. Despite this, it has only been performed in less than 20% of cases[20]. t-MN patients are often excluded as candidates for allogeneic HSCT due to poor performance status, advanced age and the lack of a matched donor. For those patients who receive a transplant, overall survival is 38% at five years and 24% at ten years[141]. Emerging data indicate that not all patients may benefit from transplantation; MDS patients with *TP53* mutations have a median overall survival of just 4.6 months after HSCT[142]. This is sobering news, as up to 40% of t-MN patients have *TP53* mutations[91]. Conversely, the addition of decitabine to transplant regimens may benefit patients with *TP53*-mutant AML and MDS[143]. However, the molecular mechanism underlying the sensitivity of *TP53*-mutated disease to decitabine is currently unclear.

Therapeutic implications

The knowledge gained in studying t-MN aetiology opens preventive and therapeutic opportunities but raises a number of new questions. The burden of rare, inherited, deleterious variants needs to be fully characterized by performing genome-wide analyses in cohorts of t-MN patients with and without a family history of cancer. Prospective trials of cancer patients are necessary to definitively ascertain the genetic risks of subsequent t-MN, with the caveat that the risk of t-MN is strongly influenced by the therapeutic regimen and will therefore change over time. These studies should determine

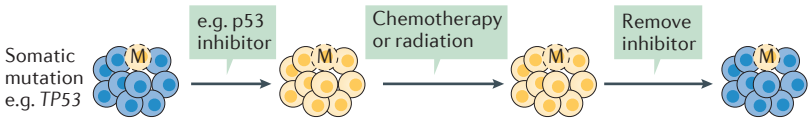


Figure 6 | **A hypothetical approach to preserve healthy haematopoietic stem cells during chemotherapy and/or radiation therapy.** A proportion of therapy-related myeloid neoplasms (t-MN) are derived from clonal selection of haematopoietic stem cells (HSCs) carrying somatic mutations (indicated by an 'M') at the expense of healthy HSCs during treatment of the primary cancer. One hypothetical approach towards preserving the healthy HSCs is to temporarily recapitulate the mutation in the healthy HSCs, such that no HSC clone has a 'fitness' advantage over the others. For example, in patients with a somatic *TP53* mutation, a p53 inhibitor administered during chemotherapy and/or radiation treatment would prevent normal HSCs from being out-competed by *TP53*-mutant HSCs. A similar strategy could be devised for other clonal haematopoiesis of indeterminate potential (CHIP) mutations.

the degree to which germline mutations in t-MN susceptibility genes correlate with subsequent t-MN.

We also need to perform prospective trials to define the extent to which CHIP can be used as a biomarker for stratifying t-MN risk. Specific questions in this area include determining the genes and individual genetic changes that are present, the clonal frequencies of these changes and the multiple gene mutations (and possibly specific combinations of mutations) that confer the highest t-MN risk. Sensitive genomic assays, such as those previously described by Kirkizlar *et al.*[144], should also be performed to identify low frequency clonal aneuploidy, as this may also prove predictive of t-MN. Ultimately, data addressing these questions should inform treatment decisions for at-risk patients at the time of the primary malignancy and provide a molecular test for long-term monitoring.

Based on the findings of such studies, therapies for the primary malignant disease could be tailored to decrease the risk of t-MN, for example, by avoiding the use or decreasing the dose of alkylating agents in genetically susceptible populations and/or through the continued reduction of radiation fields and dosages. Perhaps allogeneic HSCT donors should be screened for the presence of CHIP, and autologous HSCT should be avoided in patients with CHIP[122,145]. We also expect that non-cytotoxic therapies will be less leukaemogenic. For instance, differentiation therapy can promote maturation of cancer stem cells without eliminating healthy HSCs[146]. All-*trans* retinoic acid is a drug that induces differentiation of acute promyelocytic leukaemia (APL) cells without negatively affecting normal HSCs[146]. Based on the emerging picture of how alterations of the bone marrow niche contribute to t-MN, future chemoprevention studies targeting changes in the niche — for example, WNT signalling, inflammatory signalling or the p53-mediated stress response — to reduce the risk in individuals with CHIP may be warranted.

Given that a proportion of t-MN cases derive from the clonal selection of mutant HSCs[6,120], efforts could be directed towards preserving the healthy HSCs of patients during treatment for the primary cancer. One speculative approach to accomplish a 'clonal switch' (REF. 147) is based upon a homeopathic approach of 'like cures like'. For example, temporarily inhibiting p53 function by knocking down p53 during radiation treatment in mice[148] has the effect of 'levelling the playing field', such that normal HSCs are not out-competed by p53-deficient HSCs (FIG. 6). A similar strategy could be devised for other CHIP mutations. Another therapeutic opportunity is afforded by the high prevalence of del(5q) and –7/del(7q) abnormalities, for which the cellular requirement for a normal dosage of essential genes within the CGSs may create an opportunity to target vulnerabilities due to a reduction in the level of the protein product of a haploinsufficient gene. This situation has been demonstrated in the case of lenalidomide-induced ubiquitin-mediated degradation of casein kinase Iα (CKIα), which creates a therapeutic window for targeting the affected cells in MDS with isolated del(5q)[149–151].

Conclusions and future directions

Recent studies have provided remarkable new discoveries on the pathobiology of t-MN along the entire timeline of t-MN development. Inherited mutations in DNA repair pathways are associated with t-MN development in a small subset of patients[33–35]. Pre-existing somatic mutations acquired before treatment are selected for by chemotherapy and/or radiation[6,120]. During one or more rounds of chemotherapy, we propose that mutant leukaemia-initiating clones are selected for increased 'fitness' in response to therapy and thus are inherently chemoresistant. Altered function of the bone marrow niche may precede cytotoxic therapy, or treatment may damage the microenvironment, providing another source selecting for the outgrowth of preleukaemic clones[12–15]. Subsequent somatic mutations in t-MN are shared with other high-risk myeloid neoplasms, which is indicative of a shared aetiology[6–8]. Finally, haploinsufficiency and CGSs are now recognized as major drivers of disease[9–11]. In the future, we anticipate the discovery that other secondary cancers have comparable aetiology. Indeed, primary cancers in general likely have similar contributions from genetic predisposition, somatic clonal mosaicism (reviewed in REF. 152) and environment.

Despite these significant strides, many unanswered questions remain. It is essential to continue to classify t-MN separately from *de novo* AML so that these questions can be addressed. For example, what is the mechanism for clonal dominance? It should be determined if genes mutated in CHIP alter proliferation, quiescence, senescence, apoptosis or some other pathway to increase 'fitness'. Do specific genes mutated in CHIP confer increased fitness only in certain contexts? Why do some chemotherapeutic agents and not others promote clonal competition? Does this selectivity correlate with the extent to which the DNA damage responses are triggered? What is the full compendium of genes on chromosomes 5 and 7 involved in CGSs, and how do 5q- and 7q-encoded genes interact to drive haematopoietic transformation and promote clonal outgrowth? Is there a role for non-coding genes on 5q or 7q in addition to *mir-145* and *mir-146A*[69,72]? Do variations in the size or span of CGS deletions cause different phenotypes? What

is the sequence of genetic events in t-MN, and does the sequence order influence the disease phenotype? What are the mechanism(s) of involvement of the bone marrow niche? What is the spectrum of therapeutic targets? What is the potential for targeting the bone marrow niche or employing a combination therapy targeting both HSCs and the niche? Although these are complex problems, they are tractable. After decades of little progress in improving clinical outcomes, we now foresee a future where we can predict, prevent and treat t-MN.

1. Singh, Z. N. *et al.* Therapy-related myelodysplastic syndrome: morphologic subclassification may not be clinically relevant. *Am. J. Clin. Pathol.* **127**, 197–205 (2007).
2. Wu, S., Powers, S., Zhu, W. & Hannun, Y. A. Substantial contribution of extrinsic risk factors to cancer development. *Nature* **529**, 43–47 (2016).
3. Schrader, K. A. *et al.* Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncology* **2**, 104–111 (2016).
4. Pritchard, C. C. *et al.* Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N. Engl. J. Med.* **375**, 443–453 (2016).
5. Zhang, J. *et al.* Germline mutations in predisposition genes in pediatric cancer. *N. Engl. J. Med.* **373**, 2336–2346 (2015).
6. Wong, T. N. *et al.* Role of *TP53* mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature* **518**, 552–555 (2015).
Largest study of somatic mutations in t-MN that also reported pre-existing *TP53* mutations prior to t-MN development.
7. Ok, C. Y. *et al.* Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with *de novo* diseases. *Leuk. Res.* **39**, 348–354 (2015).
8. Lindsley, R. C. *et al.* Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* **125**, 1367–1376 (2015).
9. Stoddart, A. *et al.* Haploinsufficiency of del(5q) genes, *Egr1* and *Apc*, cooperate with *Tp53* loss to induce acute myeloid leukemia in mice. *Blood* **123**, 1069–1078 (2014).
First *in vivo* evidence of t-MN as a CGS on chromosome arm 5q.
10. McNerney, M. E. *et al. CUX1* is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. *Blood* **121**, 975–983 (2013).
11. Wong, J. C. *et al.* Functional evidence implicating chromosome 7q22 haploinsufficiency in myelodysplastic syndrome pathogenesis. *eLife* http://dx.doi.org/10.7554/eLife.07839 (2015).
12. Raaijmakers, M. H. Myelodysplastic syndromes: revisiting the role of the bone marrow microenvironment in disease pathogenesis. *Int. J. Hematol.* **95**, 17–25 (2012).
13. Agarwal, P. & Bhatia, R. Influence of bone marrow microenvironment on leukemic stem cells: breaking up an intimate relationship. *Adv. Cancer Res.* **127**, 227–252 (2015).
14. Anthony, B. A. & Link, D. C. Regulation of hematopoietic stem cells by bone marrow stromal cells. *Trends Immunol.* **35**, 32–37 (2014).
15. Korn, C. & Mendez-Ferrer, S. Myeloid malignancies and the microenvironment. *Blood* **129**, 811–822 (2016).
16. de Moor, J. S. *et al.* Cancer survivors in the United States: prevalence across the survivorship trajectory and implications for care. *Cancer Epidemiol. Biomarkers Prev.* **22**, 561–570 (2013).
17. Rowland, J. H. & Bellizzi, K. M. Cancer survivorship issues: life after treatment & implications for an aging population. *Clin. J. Oncol.* **32**, 2662–2668 (2014).
18. De Roos, A. J. *et al.* Incidence of myelodysplastic syndromes within a nonprofit healthcare system in western Washington state, 2005–2006. *Am. J. Hematol.* **85**, 765–770 (2010).
19. Hulegardh, E. *et al.* Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: a report from the Swedish Acute Leukemia Registry. *Am. J. Hematol.* **90**, 208–214 (2015).
20. Fianchi, L. *et al.* Characteristics and outcome of therapy-related myeloid neoplasms: report from the Italian network on secondary leukemias. *Am. J. Hematol.* **90**, E80–E85 (2015).
21. Morton, L. M. *et al.* Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975–2008. *Blood* **121**, 2996–3004 (2013).
22. Morton, L. M. *et al.* Risk of myeloid neoplasms after solid organ transplantation. *Leukemia* **28**, 2317–2323 (2014).
23. Radivoyevitch, T. *et al.* Defining AML and MDS second cancer risk dynamics after diagnoses of first cancers treated or not with radiation. *Leukemia* **30**, 285–294 (2016).
Provides a thorough analysis of environmental risk factors for t-MN.
24. Smith, S. M. *et al.* Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood* **102**, 43–52 (2003).
25. Wolff, A. C. *et al.* Risk of marrow neoplasms after adjuvant breast cancer therapy: the national comprehensive cancer network experience. *J. Clin. Oncol.* **33**, 340–348 (2015).
26. Eichenauer, D. A. *et al.* Therapy-related acute myeloid leukemia and myelodysplastic syndromes in patients with Hodgkin lymphoma: a report from the German Hodgkin Study Group. *Blood* **123**, 1658–1664 (2014).
27. Armitage, J. O. *et al.* Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. *J. Clin. Oncol.* **21**, 897–906 (2003).
28. Smith, M. R. *et al.* Incidence of therapy-related myeloid neoplasia after initial therapy for chronic lymphocytic leukemia with fludarabine-cyclophosphamide versus fludarabine: long-term follow-up of US Intergroup Study E2997. *Blood* **118**, 3525–3527 (2011).
29. Pemmaraju, N. *et al.* Characteristics and outcomes of patients with multiple myeloma who develop therapy-related myelodysplastic syndrome, chronic myelomonocytic leukemia, or acute myeloid leukemia. *Clin. Lymphoma Myeloma Leuk.* **15**, 110–114 (2015).
30. Bhatia, S. *et al.* Therapy-related myelodysplasia and acute myeloid leukemia after Ewing sarcoma and primitive neuroectodermal tumor of bone: a report from the Children's Oncology Group. *Blood* **109**, 46–51 (2007).
31. Ballinger, M. L. *et al.* Monogenic and polygenic determinants of sarcoma risk: an international genetic study. *Lancet Oncol.* **17**, 1261–1271 (2016).
32. Bhatia, S. Genetic variation as a modifier of association between therapeutic exposure and subsequent malignant neoplasms in cancer survivors. *Cancer* **121**, 648–663 (2015).
33. Churpek, J. E. *et al.* Inherited mutations in cancer susceptibility genes are common among survivors of breast cancer who develop therapy-related leukemia. *Cancer* **122**, 304–311 (2016).
This study demonstrates a high frequency of mutations in cancer predisposition genes in patients who developed t-MN following treatment for breast cancer.
34. Schulz, E. *et al.* Germline mutations in the DNA damage response genes *BRCA1*, *BRCA2*, *BARD1* and *TP53* in patients with therapy related myeloid neoplasms. *J. Med. Genet.* **49**, 422–428 (2012).
35. Voso, M. T. *et al.* Fanconi anemia gene variants in therapy-related myeloid neoplasms. *Blood Cancer J.* **5**, e323 (2015).
36. Felix, C. A. *et al.* The *p53* gene in pediatric therapy-related leukemia and myelodysplasia. *Blood* **87**, 4376–4381 (1996).
37. Felix, C. A. *et al.* Association of germline *p53* mutation with MLL segmental jumping translocation in treatment-related leukemia. *Blood* **91**, 4451–4456 (1998).
38. Link, D. C. *et al.* Identification of a novel *TP53* cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML. *JAMA* **305**, 1568–1576 (2011).
39. Momota, H. *et al.* Acute lymphoblastic leukemia after temozolomide treatment for anaplastic astrocytoma in a child with a germline TP53 mutation. *Pediatr. Blood Cancer* **55**, 577–579 (2010).
40. Vasanthakumar, A. *et al.* Brca1 deficiency causes bone marrow failure and spontaneous hematologic malignancies in mice. *Blood* **127**, 310–313 (2016).
41. Ramus, S. J. *et al.* Increased frequency of *TP53* mutations in BRCA1 and BRCA2 ovarian tumours. *Genes Chromosomes Cancer* **25**, 91–96 (1999).
42. Schuyer, M. & Berns, E. M. Is TP53 dysfunction required for BRCA1-associated carcinogenesis? *Mol. Cell. Endocrinol.* **155**, 143–152 (1999).
43. Jacoby, M. A. *et al.* The DNA double-strand break response is abnormal in myeloblasts from patients with therapy-related acute myeloid leukemia. *Leukemia* **28**, 1242–1251 (2014).
44. Maris, J. M. *et al.* Monosomy 7 myelodysplastic syndrome and other second malignant neoplasms in children with neurofibromatosis type 1. *Cancer* **79**, 1438–1446 (1997).
45. Chao, R. C. *et al.* Therapy-induced malignant neoplasms in Nf1 mutant mice. *Cancer Cell* **8**, 337–348 (2005).
46. Choi, G. *et al.* Genetically mediated Nf1 loss in mice promotes diverse radiation-induced tumors modeling second malignant neoplasms. *Cancer Res.* **72**, 6425–6434 (2012).
47. Greene, M. H. *et al.* Melphalan may be a more potent leukemogen than cyclophosphamide. *Ann. Intern. Med.* **105**, 360–367 (1986).
48. Curtis, R. E. *et al.* Risk of leukemia after chemotherapy and radiation treatment for breast cancer. *N. Engl. J. Med.* **326**, 1745–1751 (1992).
49. Travis, L. B. *et al.* Risk of leukemia after platinum-based chemotherapy for ovarian cancer. *N. Engl. J. Med.* **340**, 351–357 (1999).
50. Travis, L. B. *et al.* Treatment-associated leukemia following testicular cancer. *J. Natl Cancer Inst.* **92**, 1165–1171 (2000).
51. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. *N. Engl. J. Med.* **368**, 2059–2074 (2013).
52. Offman, J. *et al.* Defective DNA mismatch repair in acute myeloid leukemia/myelodysplastic syndrome after organ transplantation. *Blood* **104**, 822–828 (2004).
53. Waterman, J. *et al.* Fludarabine as a risk factor for poor stem cell harvest, treatment-related MDS and AML in follicular lymphoma patients after autologous hematopoietic cell transplantation. *Bone Marrow Transplant.* **47**, 488–493 (2012).
54. Kim, G. *et al.* FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. *Clin. Cancer Res.* **21**, 4257–4261 (2015).
55. Lange, R. D., Moloney, W. C. & Yamawaki, T. Leukemia in atomic bomb survivors. I. General observations. *Blood* **9**, 574–585 (1954).
56. Travis, L. B. *et al.* Second malignant neoplasms and cardiovascular disease following radiotherapy. *J. Natl Cancer Inst.* **104**, 357–370 (2012).
57. Nardi, V. *et al.* Acute myeloid leukemia and myelodysplastic syndromes after radiation therapy are similar to *de novo* disease and differ from other therapy-related myeloid neoplasms. *J. Clin. Oncol.* **30**, 2340–2347 (2012).
58. Mukherjee, S. *et al.* Risk for developing myelodysplastic syndromes in prostate cancer patients definitively treated with radiation. *J. Natl. Cancer Inst.* **106**, djt462 (2014).
59. Bello, C. *et al.* Outcomes after induction chemotherapy in patients with acute myeloid leukemia arising from myelodysplastic syndrome. *Cancer* **117**, 1463–1469 (2011).
60. Appelbaum, F. R. *et al.* Age and acute myeloid leukemia. *Blood* **107**, 3481–3485 (2006).
61. Ernst, T. *et al.* Inactivating mutations of the histone methyltransferase gene *EZH2* in myeloid disorders. *Nat. Genet.* **42**, 722–726 (2010).

62. Lai, F. *et al.* Transcript map and comparative analysis of the 1.5-Mb commonly deleted segment of human 5q31 in malignant myeloid diseases with a del(5q). *Genomics* **71**, 235–245 (2001).
63. Le Beau, M. M. *et al.* Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. *Proc. Natl Acad. Sci. USA* **90**, 5484–5488 (1993).
64. Le Beau, M. M. *et al.* Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases. *Blood* **88**, 1930–1935 (1996).
65. Boultwood, J. *et al.* Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. *Blood* **99**, 4638–4641 (2002).
66. Jerez, A. *et al.* Topography, clinical, and genomic correlates of 5q myeloid malignancies revisited. *J. Clin. Oncol.* **30**, 1343–1349 (2012).
67. Wang, J., Fernald, A. A., Anastasi, J., Le Beau, M. M. & Qian, Z. Haploinsufficiency of *Apc* leads to ineffective hematopoiesis. *Blood* **115**, 3481–3488 (2010).
68. Ebert, B. L. *et al.* Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. *Nature* **451**, 335–339 (2008).
69. Starczynowski, D. T. *et al.* Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat. Med.* **16**, 49–58 (2010).
70. Min, I. M. *et al.* The transcription factor EGR1 controls both the proliferation and localization of hematopoietic stem cells. *Cell Stem Cell* **2**, 380–391 (2008).
71. Schneider, R. K. *et al.* Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. *Cancer Cell* **26**, 509–520 (2014).
72. Kumar, M. S. *et al.* Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q- syndrome. *Blood* **118**, 4666–4673 (2011).
73. Stoddart, A., Nakitandwe, J., Chen, S. C., Downing, J. R. & Le Beau, M. M. Haploinsufficient loss of multiple 5q genes may fine-tune Wnt signaling in del(5q) therapy-related myeloid neoplasms. *Blood* **126**, 2899–2901 (2015).
74. Schmickel, R. D. Contiguous gene syndromes: a component of recognizable syndromes. *J. Pediatr.* **109**, 231–241 (1986).
75. Liu, Y. *et al.* Deletions linked to *TP53* loss drive cancer through p53-independent mechanisms. *Nature* **531**, 471–475 (2016).
Demonstration of a CGS on chromosome arm 17p.
76. Parker, H. *et al.* 13q deletion anatomy and disease progression in patients with chronic lymphocytic leukemia. *Leukemia* **25**, 489–497 (2011).
77. Tseng, Y. Y. *et al.* PVT1 dependence in cancer with MYC copy-number increase. *Nature* **512**, 82–86 (2014).
78. Solimini, N. L. *et al.* Recurrent hemizygous deletions in cancers may optimize proliferative potential. *Science* **337**, 104–109 (2012).
79. Wong, C. C. *et al.* Inactivating *CUX1* mutations promote tumorigenesis. *Nat. Genet.* **46**, 33–38 (2014).
80. Hosono, N. *et al.* Recurrent genetic defects on chromosome 7q in myeloid neoplasms. *Leukemia* **28**, 1348–1351 (2014).
81. Chen, C. *et al.* MLL3 is a haploinsufficient 7q tumor suppressor in acute myeloid leukemia. *Cancer Cell* **25**, 652–665 (2014).
82. Nagamachi, A. *et al.* Haploinsufficiency of *SAMD9L*, an endosome fusion facilitator, causes myeloid malignancies in mice mimicking human diseases with monosomy 7. *Cancer Cell* **24**, 305–317 (2013).
83. Chen, D. H. *et al.* Ataxia-pancytopenia syndrome Is caused by missense mutations in *SAMD9L*. *Am. J. Hum. Genet.* **98**, 1146–1158 (2016).
84. Minelli, A. *et al.* The isochromosome i(7)(q10) carrying c.258 + 2t > c mutation of the *SBDS* gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia* **23**, 708–711 (2009).
85. Narumi, S. *et al. SAMD9* mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. *Nat. Genet.* **48**, 792–797 (2016).
86. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
87. McNerney, M. E. *et al.* The spectrum of somatic mutations in high-risk acute myeloid leukaemia with −7/del(7q). *Br. J. Haematol.* **166**, 550–556 (2014).
88. Hunter, C. *et al.* A hypermutation phenotype and somatic *MSH6* mutations in recurrent human malignant gliomas after alkylator chemotherapy. *Cancer Res.* **66**, 3987–3991 (2006).
89. Mistry, A. R. *et al.* DNA topoisomerase II in therapy-related acute promyelocytic leukemia. *N. Engl. J. Med.* **352**, 1529–1538 (2005).
90. Kondo, N., Takahashi, A., Ono, K. & Ohnishi, T. DNA damage induced by alkylating agents and repair pathways. *J. Nucleic Acids* **2010**, 543531 (2010).
91. Ok, C. Y. *et al.* TP53 mutation characteristics in therapy-related myelodysplastic syndromes and acute myeloid leukemia is similar to *de novo* diseases. *J. Hematol. Oncol.* **8**, 45 (2015).
92. Christiansen, D. H., Andersen, M. K. & Pedersen-Bjergaard, J. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia & acute myeloid leukemia after exposure to alkylating agents & significantly associated with deletion or loss of 5q, a complex karyotype and a poor prognosis. *J. Clin. Oncol.* **19**, 1405–1413 (2001).
93. Side, L. E. *et al. RAS*, *FLT3*, and *TP53* mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. *Genes Chromosomes Cancer* **39**, 217–223 (2004).
94. Cox, A. D., Fesik, S. W., Kimmelman, A. C., Luo, J. & Der, C. J. Drugging the undruggable RAS: mission possible? *Nat. Rev. Drug Discov.* **13**, 828–851 (2014).
95. Schulz, E. *et al.* Preexisting *TP53* mutation in therapy-related acute myeloid leukemia. *Ann. Hematol.* **94**, 527–529 (2015).
96. Adem, V. *et al.* The complete mutatome and clonal architecture of del(5q) [Abstract 608]. (American Society of Hematology Annual Meeting, 2015).
97. Woll, P. S. *et al.* Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells *in vivo*. *Cancer Cell* **25**, 794–808 (2014).
98. Dumitriu, B. *et al.* Telomere attrition and candidate gene mutations preceding monosomy 7 in aplastic anemia. *Blood* **125**, 706–709 (2015).
99. Cachia, P. G. *et al.* Clonal haemopoiesis following cytotoxic therapy for lymphoma. *Leukemia* **7**, 795–800 (1993).
100. Ruark, E. *et al.* Mosaic *PPM1D* mutations are associated with predisposition to breast and ovarian cancer. *Nature* **493**, 406–410 (2013).
101. Swisher, E. M. *et al.* Somatic mosaic mutations in *PPM1D* and *TP53* in the blood of women with ovarian carcinoma. *JAMA Oncology* **2**, 370–372 (2016).
102. Pharoah, P. D. *et al. PPM1D* mosaic truncating variants in ovarian cancer cases may be treatment-related somatic mutations. *J. Natl. Cancer Inst.* **108**, djv347 (2016).
103. Steensma, D. P. *et al.* Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126**, 9–16 (2015).
104. Rodriguez-Santiago, B. *et al.* Mosaic uniparental disomies and aneuploidies as large structural variants of the human genome. *Am. J. Hum. Genet.* **87**, 129–138 (2010).
105. Forsberg, L. A. *et al.* Age-related somatic structural changes in the nuclear genome of human blood cells. *Am. J. Hum. Genet.* **90**, 217–228 (2012).
106. Jacobs, K. B. *et al.* Detectable clonal mosaicism and its relationship to aging and cancer. *Nat. Genet.* **44**, 651–658 (2012).
107. Laurie, C. C. *et al.* Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat. Genet.* **44**, 642–650 (2012).
108. Machiela, M. J. *et al.* Characterization of large structural genetic mosaicism in human autosomes. *Am. J. Hum. Genet.* **96**, 487–497 (2015).
109. Schick, U. M. *et al.* Confirmation of the reported association of clonal chromosomal mosaicism with an increased risk of incident hematologic cancer. *PLoS ONE* **8**, e59823 (2013).
110. Genovese, G. *et al.* Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* **371**, 2477–2487 (2014).
111. Jaiswal, S. *et al.* Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* **371**, 2488–2498 (2014).
112. Xie, M. *et al.* Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* **20**, 1472–1478 (2014).
113. McKerrell, T. *et al.* Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep.* **10**, 1239–1245 (2015).
114. Young, A. L., Challen, G. A., Birmann, B. M. & Druley, T. E. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat. Commun.* **7**, 12484 (2016).
115. Rossi, L. *et al.* Less is more: unveiling the functional core of hematopoietic stem cells through knockout mice. *Cell Stem Cell* **11**, 302–317 (2012).
116. Moran-Crusio, K. *et al.* Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20**, 11–24 (2011).
117. Challen, G. A. *et al.* Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* **44**, 23–31 (2012).
118. Shlush, L. I. *et al.* Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* **506**, 328–333 (2014).
119. Bondar, T. & Medzhitov, R. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* **6**, 309–322 (2010).
120. Takahashi, K. *et al.* Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *Lancet Oncol.* **18**, 100–111 (2017).
121. Gillis, N. K. *et al.* Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol.* **18**, 112–121 (2017).
122. Gibson, C. J. *et al.* Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. *J. Clin. Oncol.* **35**, 1598–1605 (2017).
References 120–122 demonstrate CHIP as a potential biomarker for t-MN.
123. Yoshizato, T. *et al.* Somatic mutations and clonal hematopoiesis in aplastic anemia. *N. Engl. J. Med.* **373**, 35–47 (2015).
124. Babushok, D. V. *et al.* Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. *Cancer Genet.* **208**, 115–128 (2015).
125. Maciejewski, J. P., Risitano, A., Sloand, E. M., Nunez, O. & Young, N. S. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. *Blood* **99**, 3129–3135 (2002).
126. Kfoury, Y. & Scadden, D. T. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* **16**, 239–253 (2015).
127. Ferrer, R. A. *et al.* Mesenchymal stromal cells from patients with myelodyplastic syndrome display distinct functional alterations that are modulated by lenalidomide. *Haematologica* **98**, 1677–1685 (2013).
128. Medyouf, H. *et al.* Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell* **14**, 824–837 (2014).
This study showed that patient-derived MSCs promote MDS stem cell growth in mouse xenografts.
129. Raaijmakers, M. H. *et al.* Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature* **464**, 852–857 (2010).
130. Stoddart, A. *et al.* Cell intrinsic and extrinsic factors synergize in mice with haploinsufficiency for *Tp53*, and two human del(5q) genes, *Egr1* and *Apc*. *Blood* **123**, 228–238 (2014).
131. Fleming, H. E. *et al.* Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal *in vivo*. *Cell Stem Cell* **2**, 274–283 (2008).
132. Wang, Y. *et al.* The Wnt/β-catenin pathway is required for the development of leukemia stem cells in AML. *Science* **327**, 1650–1653 (2010).
133. Lane, S. W. *et al.* The Apc^{min} mouse has altered hematopoietic stem cell function and provides a model for MPD/MDS. *Blood* **115**, 3489–3497 (2010).
134. Falconi, G. *et al.* Impairment of PI3K/AKT and WNT/β-catenin pathways in bone marrow mesenchymal stem cells isolated from patients with myelodysplastic syndromes. *Exp. Hematol.* **44**, 75–83e71-74 (2016).
135. Kode, A. *et al.* Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. *Nature* **506**, 240–244 (2014).
136. Zambetti, N. A. *et al.* Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human pre-leukemia. *Cell Stem Cell* **19**, 613–627 (2016).
137. Link, D. C. & Walter, M. J. 'CHIP'ping away at clonal hematopoiesis. *Leukemia* **30**, 1633–1635 (2016).
138. Granfeldt Ostgard, L. S. *et al.* Epidemiology and clinical significance of secondary and therapy-related acute myeloid leukemia: a national population-based cohort study. *J. Clin. Oncol.* **33**, 3641–3649 (2015).

139. Borthakur, G. *et al.* Survival is poorer in patients with secondary core-binding factor acute myelogenous leukemia compared with *de novo* core-binding factor leukemia. *Cancer* **115**, 3217–3221 (2009).
140. Kayser, S. *et al.* The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. *Blood* **117**, 2137–2145 (2011).
141. Finke, J. *et al.* Long-term follow-up of therapy-related myelodysplasia and AML patients treated with allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant.* **51**, 771–777 (2016).
142. Bejar, R. *et al.* Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. *J. Clin. Oncol.* **32**, 2691–2698 (2014).
143. Welch, J. S. *et al.* TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N. Engl. J. Med.* **375**, 2023–2036 (2016).
144. Kirkizlar, E. *et al.* Detection of clonal and subclonal copy-number variants in cell-free DNA from patients with breast cancer using a massively multiplexed PCR methodology. *Transl Oncol.* **8**, 407–416 (2015).
145. Rojek, K. *et al.* Identifying inherited and acquired genetic factors involved in poor stem cell mobilization and donor-derived malignancy. *Biol. Blood Marrow Transplant.* **22**, 2100–2103 (2016).
146. Collins, S. J. The role of retinoids and retinoic acid receptors in normal hematopoiesis. *Leukemia* **16**, 1896–1905 (2002).
147. Goodell, M. A. & Rando, T. A. Stem cells and healthy aging. *Science* **350**, 1199–1204 (2015).
148. Lee, C. L. *et al.* Acute DNA damage activates the tumour suppressor p53 to promote radiation-induced lymphoma. *Nat. Communications* **6**, 8477 (2015).
149. Frei, E. 3rd. Gene deletion: a new target for cancer chemotherapy. *Lancet* **342**, 662–664 (1993).
150. Jaras, M. *et al.* Csnk1a1 inhibition has p53-dependent therapeutic efficacy in acute myeloid leukemia. *J. Exp. Med.* **211**, 605–612 (2014).
151. Kronke, J. *et al.* Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. *Nature* **523**, 183–188 (2015).
This study demonstrates that a CGS can provide a therapeutic targeting opportunity.
152. Fernandez, L. C., Torres, M. & Real, F. X. Somatic mosaicism: on the road to cancer. *Nat. Rev. Cancer* **16**, 43–55 (2016).
153. Shih, A. H. *et al.* Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia. *Haematologica* **98**, 908–912 (2013).
154. Christiansen, D. H., Andersen, M. K., Desta, F. & Pedersen-Bjergaard, J. Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia* **19**, 2232–2240 (2005).
155. Voso, M. T. *et al.* Mutations of epigenetic regulators and of the spliceosome machinery in therapy-related myeloid neoplasms and in acute leukemias evolved from chronic myeloproliferative diseases. *Leukemia* **27**, 982–985 (2013).
156. Desta, F., Christiansen, D. H., Andersen, M. K. & Pedersen-Bjergaard, J. Activating mutations of *JAK2V617F* are uncommon in t-MDS and t-AML and are only observed in atypic cases. *Leukemia* **20**, 547–548 (2006).
157. Bacher, U., Haferlach, T., Kern, W., Haferlach, C. & Schnittger, S. A comparative study of molecular mutations in 381 patients with myelodysplastic syndrome and in 4130 patients with acute myeloid leukemia. *Haematologica* **92**, 744–752 (2007).
158. Andersen, M. T., Andersen, M. K., Christiansen, D. H. & Pedersen-Bjergaard, J. *NPM1* mutations in therapy-related acute myeloid leukemia with uncharacteristic features. *Leukemia* **22**, 951–955 (2008).
159. Christiansen, D. H., Desta, F., Andersen, M. K. & Pedersen-Bjergaard, J. Mutations of the *PTPN11* gene in therapy-related MDS and AML with rare balanced chromosome translocations. *Genes Chromosomes Cancer* **46**, 517–521 (2007).
160. Christiansen, D. H., Andersen, M. K. & Pedersen-Bjergaard, J. Mutations of *AML1* are common in therapy-related myelodysplasia following therapy with alkylating agents and are significantly associated with deletion or loss of chromosome arm 7q and with subsequent leukemic transformation. *Blood* **104**, 1474–1481 (2004).
161. Fabiani, E. *et al. SETBP1* mutations in 106 patients with therapy-related myeloid neoplasms. *Haematologica* **99**, e152–e153 (2014).
162. Grimwade, D. *et al.* 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**, 1312–1320 (2001).
163. Frohling, S. *et al.* 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**, 3280–3288 (2006).
164. Grimwade, D. *et al.* Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* **116**, 354–365 (2010).
165. Chou, W. C. *et al.* Distinct clinical and biological features of *de novo* acute myeloid leukemia with additional sex comb-like 1 (*ASXL1*) mutations. *Blood* **116**, 4086–4094 (2010).
166. Barjesteh van Waalwijk van Doorn-Khosrovani, S. *et al.* Somatic heterozygous mutations in ETV6 (TEL) and frequent absence of ETV6 protein in acute myeloid leukemia. *Oncogene* **24**, 4129–4137 (2005).
167. Schnittger, S. *et al.* Analysis of *FLT3* length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood* **100**, 59–66 (2002).
168. Paschka, P. *et al. IDH1* and *IDH2* mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *J. Clin. Oncol.* **28**, 3636–3643 (2010).
169. Levine, R. L. *et al.* The *JAK2V617F* activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood* **106**, 3377–3379 (2005).
170. Steudel, C. *et al.* Comparative analysis of *MLL* partial tandem duplication and *FLT3* internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia. *Genes Chromosomes Cancer* **37**, 237–251 (2003).
171. Falini, B. *et al.* Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N. Engl. J. Med.* **352**, 254–266 (2005).
172. Bacher, U., Haferlach, T., Schoch, C., Kern, W. & Schnittger, S. Implications of *NRAS* mutations in AML: a study of 2502 patients. *Blood* **107**, 3847–3853 (2006).
173. Van Vlierberghe, P. *et al. PHF6* mutations in adult acute myeloid leukemia. *Leukemia* **25**, 130–134 (2011).
174. Thol, F. *et al.* Mutations in the cohesin complex in acute myeloid leukemia: clinical and prognostic implications. *Blood* **123**, 914–920 (2014).
175. Gaidzik, V. I. *et al. RUNX1* mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. *J. Clin. Oncol.* **29**, 1364–1372 (2011).
176. Tang, J. L. *et al. AML1/RUNX1* mutations in 470 adult patients with *de novo* acute myeloid leukemia: prognostic implication and interaction with other gene alterations. *Blood* **114**, 5352–5361 (2009).
177. Thol, F. *et al. SETBP1* mutation analysis in 944 patients with MDS and AML. *Leukemia* **27**, 2072–2075 (2013).
178. Papaemmanuil, E. *et al.* Somatic *SF3B1* mutation in myelodysplasia with ring sideroblasts. *N. Engl. J. Med.* **365**, 1384–1395 (2011).
179. Chou, W. C. *et al. TET2* mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. *Blood* **118**, 3803–3810 (2011).
180. Weissmann, S. *et al.* Landscape of *TET2* mutations in acute myeloid leukemia. *Leukemia* **26**, 934–942 (2012).
181. Grossmann, V. *et al.* A novel hierarchical prognostic model of AML solely based on molecular mutations. *Blood* **120**, 2963–2972 (2012).
182. Patel, J. P. *et al.* Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N. Engl. J. Med.* **366**, 1079–1089 (2012).
183. Hou, H. A. *et al. DNMT3A* mutations in acute myeloid leukemia: stability during disease evolution and clinical implications. *Blood* **119**, 559–568 (2012).
184. Dohner, K. *et al.* Molecular cytogenetic characterization of a critical region in bands 7q35-q36 commonly deleted in malignant myeloid disorders. *Blood* **92**, 4031–4035 (1998).
185. Jerez, A. *et al.* Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. *Blood* **119**, 6109–6117 (2012).
186. Varney, M. E. *et al.* Loss of *Tifab*, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor-TRAF6 signaling. *J. Exp. Med.* **212**, 1967–1985 (2015).
187. Joslin, J. M. *et al.* Haploinsufficiency of *EGR1*, a candidate gene in the del(5q), leads to the development of myeloid disorders. *Blood* **110**, 719–726 (2007).
188. Young, D. J. *et al.* Knockdown of *Hnrnpa0*, a del(5q) gene, alters myeloid cell fate in murine cells through regulation of AU-rich transcripts. *Haematologica* **99**, 1032–1040 (2014).
189. Chen, T. H. *et al.* Knockdown of *Hspa9*, a del(5q31.2) gene, results in a decrease in hematopoietic progenitors in mice. *Blood* **117**, 1530–1539 (2011).
190. Peng, J. *et al.* Myeloproliferative defects following targeting of the *Drf1* gene encoding the mammalian diaphanous related formin mDia1. *Cancer Res.* **67**, 7565–7571 (2007).
191. Zhao, Z. *et al.* Cooperative loss of RAS feedback regulation drives myeloid leukemogenesis. *Nat. Genet.* **47**, 539–543 (2015).
192. Yoshimi, A. *et al.* Recurrent *CDC25C* mutations drive malignant transformation in FPD/AML. *Nat. Communications* **5**, 4770 (2014).
193. Liu, T. X. *et al.* Chromosome 5q deletion and epigenetic suppression of the gene encoding α-catenin (*CTNNA1*) in myeloid cell transformation. *Nat. Med.* **13**, 78–83 (2007).
194. Lehmann, S. *et al.* Common deleted genes in the 5q-syndrome: thrombocytopenia and reduced erythroid colony formation in SPARC null mice. *Leukemia* **21**, 1931–1936 (2007).
195. Heuser, M. *et al.* Loss of MLL5 results in pleiotropic hematopoietic defects, reduced neutrophil immune function, and extreme sensitivity to DNA demethylation. *Blood* **113**, 1432–1443 (2009).
196. Madan, V. *et al.* Impaired function of primitive hematopoietic cells in mice lacking the Mixed-Lineage-Leukemia homolog MLL5. *Blood* **113**, 1444–1454 (2009).
197. Zhang, Y. *et al.* MLL5 contributes to hematopoietic stem cell fitness and homeostasis. *Blood* **113**, 1455–1463 (2009).
198. Sundaravel, S. *et al.* Reduced DOCK4 expression leads to erythroid dysplasia in myelodysplastic syndromes. *Proc. Natl Acad. Sci. USA* **112**, E6359–E6368 (2015).
199. Muto, T. *et al.* Concurrent loss of *Ezh2* and *Tet2* cooperates in the pathogenesis of myelodysplastic disorders. *J. Exp. Med.* **210**, 2627–2639 (2013).
200. Lawrence, M. S. *et al.* Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **505**, 495–501 (2014).
201. Bowler, T. G. *et al.* Exome sequencing of familial MDS reveals novel mutations and high rates of false positive mutations in MLL3 due to pseudogene effects (Abstract #4591). (American Society of Hematology Annual Meeting, 2015).

Acknowledgements
The authors thank Angela Stoddart and Kevin M. Shannon for critical reading of the manuscript. M.E.M. is supported by NIH 1K08CA181254, The V Foundation for Cancer Research (V Foundation Scholar Award), the University of Chicago Medicine Comprehensive Cancer Center CCSG (P30 CA14599), an Institutional Research Grant (IRG-58-004-53-IRG) from the American Cancer Society and the University of Chicago Cancer Research Foundation Auxiliary Board. L.A.G. is supported by grants from the Edward P. Evans Foundation, the Taub Foundation, the Leukemia and Lymphoma Society and the Cancer Research Foundation. M.M.L. is supported by grants from NIH (CA190372) and the Edward P. Evans Foundation.

Author contributions
M.E.M., L.A.G. and M.M.L. conceived of, wrote and edited the manuscript.

Competing interests statement
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.