Towards personalised medicine for cancer

From initial therapy to follow-up

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Chapter 15: Myeloid neoplasia mutation rates differ in primary cases versus cases arising after cancers treated or not with radiation and/or chemotherapy


Abstract
Therapy-related myeloid neoplasms (tMN) associate with complex karyotypes, chromosome 7q loss, and TP53 mutations. Age-related primary (p)MN associate more with specific point mutations. Possible explanations are that background processes are likely more diverse and persistent than therapeutic agent exposures, and diversity may be needed to produce specific DNA lesions needed to produce a specific point mutation, and persistence may be needed to produce these lesions in rare moments of haematopoietic stem cell replication. Our goal was to determine if sequencing data from MN patients supports this claim. We sequenced ~60 genes often mutated in MN in 721 pMN, 125 tMN, and 48 unexposed second cancer MN (uMN, i.e. the first cancer was not treated with cytotoxic agents). Of the tMN, 45 were treated with both radiotherapy and chemotherapy, 43 with chemotherapy only, and 37 with radiotherapy only. Comparing tMN versus pMN yielded SF3B1 mutations in 2% versus 10%, \( P = 0.0009 \), JAK2 mutations in 1% versus 7%, \( P = 0.0025 \), RAS family genes (NRAS/NF1/KRAS/PTPN1/CBL) mutations in 5% versus 15%, \( P = 0.0015 \), loss of chromosome 7/7q in 28% versus 12%, \( P < 0.0001 \), and TP53 mutations in 11.2% versus 5.4%, \( P = 0.03 \). Analyses of chemotherapy and radiotherapy separately yielded similar results, save chemotherapy associating more with TP53 mutations at 15% versus 5%, \( P = 0.002 \) and radiotherapy more with EZH2 mutations at 11% versus 4.1%, \( P = 0.01 \). Comparing uMN versus pMN yielded PHF6 mutations in 10% versus 2%, \( P = 0.006 \) and IDH1 mutations in 10% versus 2%, \( P = 0.009 \). Intriguingly, however, uMN versus tMN similarly yielded IDH1 mutations in 10% versus 1%, \( P = 0.007 \). In conclusion, specific point mutations, such as SF3B1 mutations and JAK2 mutations, are not produced by cancer treatments. Relative to radiotherapy, chemotherapy provides more persistent systemic selective pressure for TP53 mutations expansion. EZH2 mutations are radiation-induced. PHF6 mutations and IDH1 mutations associate with other factors correlating first and second cancers. Regarding the latter, cancer treatments may be coincidentally suppressing or destroying subclinical IDH1 mutations clones of haematopoietic cells.

Introduction
Risks of acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS) rise and fall after diagnoses of prior cancers, particularly if they are treated with ionizing radiation.\(^\text{489}\) The significance of this may be rising, as survival improvements imply that the cohort of cancer survivors is increasing, which means that the number of person-years at risk of developing tMN are increasing as well. It has been estimated that ~7% of AML cases are therapy-related.\(^\text{490}\) Pooling AML and MDS as MN, our goal was to find genes differentially mutated in pMN versus MN after cancers treated with chemotherapy, radiotherapy, both, or neither. Those with neither are unexposed and are thus uMN. This group includes those treated with surgery alone.

Mutation rate differences in pMN versus tMN are expected. For example, the SF3B1\(^{K700E}\) mutation (SF3B1\(^{A2098G}\) (T>C) is found increasingly with age in MN\(^\text{491}\) and in healthy elderly with clonal haematopoiesis of indeterminate potential (CHIP),\(^\text{492,493}\) so a background process exists that produces it, but it likely requires DNA replication across a specific type of base damage in a very slowly dividing haematopoietic stem cell (HSC), and transient therapeutic exposures to specific agents may not be capable of creating the type of damage needed, nor the persistency for it to exist during HSC DNA replication. In contrast, background mutagenic processes may be diverse enough in
damage types, and also persistent enough to strike replicating HSC, to cause such point mutations. Indeed, the frequent occurrence of SF3B1^K700E in pMN and CHIP proves this for T>C mutations. There is thus a basis for expecting specific point mutations to associate more with age-related pMN than with therapy-related tMN. We provide MN DNA sequencing results that support this view. Our results also identify genes associated with correlations between first and second cancers due to factors other than treatment of the first cancer.

Methods

We sequenced DNA from 894 MN cases (Figure 1). Of these 52 were whole exomes using a HiSeq 2000 (Illumina) and 842 were targeted to genes frequently mutated in MN (APC, ASXL1, BCOR, BCORL1, C7orf55, CBL, CUX1, DDX41, DDX54, DHX29, DNMT3A, ETV6, EZH2, GPR98, IDH1, IDH2, JAK2, KDM6A, KRAS, MECOM, NF1, NPM1, NRAS, PHF6, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, ZRSR2) using Illumina MiSeq technology. Variants were called using the Broad Institute’s GATK Best Practices. Fisher’s exact test was used to compute P values for differences in proportions and odds ratios (OR) and their 95% confidence intervals (CI). The R package forestplot was used to generate forest plots of such OR. All computations were performed in R. P values were two-sided with α = 0.05.

Results

Study Population. We studied 894 MN cases. Of these, 721 were pMN, 125 were tMN, and 48 were uMN (Figure 1). Of cancers arising before tMN, 45 were treated with both chemotherapy and radiotherapy, 43 with only chemotherapy, and 37 with only radiotherapy. Most cancer arising before uMN were treated for their previous cancers with surgery alone. As lymphoid neoplasia (LN) tends to be systemic and thus treated with chemotherapy, uMN disproportionately lack LN as prior cancers (Figure 1). That AML cases in our cohort were mostly secondary is suggested by mutation rates in TET2 and SRSF2 being similar in MDS and AML (Table 1); this is not the case for SF3B1 mutations because such cases progresses slowly enough that most are detected as MDS.

tMN versus pMN. Comparing tMN versus pMN, SF3B1 was mutated in 2/125 (2%) versus 73/721 (10%), P = 0.0009, JAK2 was mutated in 1/125 (1%) versus 52/721 (7%), P = 0.003, RAS family genes (NRAS/NF1/KRAS/PTPN1/CBL) were mutated in 6/125 (5%) versus 105/721 (15%), P = 0.002 and this statistical difference was driven mostly by NRAS/NF1. Chromosome arm 7q was lost in 34/122 (28%) versus 80/656 (12%), P < 0.0001, and TP53 was mutated in 14/125 (11.2%) versus 39/721 (5.4%), P = 0.03 (Figure 2A). Studying chemotherapy and radiotherapy separately largely reproduced these results, save differences in TP53 and EZH2: for chemotherapy versus no chemotherapy (Figure 2B) TP53 was mutated in 13/88 (15%) versus 43/806 (5%), P = 0.002 and EZH2 mutation rates did not differ at 4/88 (5%) versus 38/806 (5%), P = 1.00, and for radiotherapy versus no radiotherapy (Figure 2C) TP53 mutation rates did not differ significantly at 8/82 (9.8%) versus 48/812 (5.9%), P = 0.23, but EZH2 rates did differ at 9/82 (11%) versus 33/812 (4.1%), P=0.01. EZH2 mutation types did not differ significantly for radiotherapy versus no radiotherapy (Supplementary Table 1).

Figure 1. Overview of the study population.

894 MN (AML + MDS) consist of 721 pMN, 125 tMN and 48 uMN. tMN include 45 patients with chemotherapy and radiotherapy, 43 with chemotherapy only, and 37 with radiotherapy only; the 48 uMN had neither. A breakdown of tMN and uMN by first cancer type is also shown. Abbreviations: AML, acute myeloid leukaemia; CT, chemotherapy; MDS, myelodysplastic syndromes; MN, myeloid neoplasm; RT, radiotherapy; pMN, primary myeloid neoplasm; tMN, treatment-related myeloid neoplasm; uMN, treatment-unrelated myeloid neoplasm; WES, whole-exome sequencing.
Table 1. Mutation frequencies of TET2, SRSF2, ASXL1 and SF3B1 in various myeloid malignancies.

<table>
<thead>
<tr>
<th></th>
<th>TET2</th>
<th>SRSF2</th>
<th>ASXL1</th>
<th>SF3B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>41/278 (15%)</td>
<td>25/279 (9%)</td>
<td>18/276 (7%)</td>
<td>5/279 (2%)</td>
</tr>
<tr>
<td>MDS</td>
<td>102/615 (17%)</td>
<td>77/615 (13%)</td>
<td>83/611 (14%)</td>
<td>75/615 (12%)</td>
</tr>
<tr>
<td>pMN</td>
<td>116/720 (16%)</td>
<td>80/721 (11%)</td>
<td>81/715 (11%)</td>
<td>73/721 (10%)</td>
</tr>
<tr>
<td>tMN</td>
<td>17/125 (14%)</td>
<td>13/125 (10%)</td>
<td>12/124 (10%)</td>
<td>2/125 (2%)</td>
</tr>
<tr>
<td>uMN (all cases)</td>
<td>10/48 (21%)</td>
<td>9/48 (19%)</td>
<td>8/48 (17%)</td>
<td>5/48 (10%)</td>
</tr>
<tr>
<td>uMN (males)</td>
<td>9/32 (28%)</td>
<td>9/32 (28%)</td>
<td>7/32 (22%)</td>
<td>2/32 (6%)</td>
</tr>
<tr>
<td>WES</td>
<td>15/52 (29%)</td>
<td>9/52 (17%)</td>
<td>8/52 (15%)</td>
<td>5/52 (10%)</td>
</tr>
</tbody>
</table>

Higher rates in uMN than pMN may reflect older ages of uMN and proportions increasing with age. \(^{491}\) Abbreviations: AML, acute myeloid leukaemia; MDS, myelodysplastic syndromes; pMN, primary myeloid neoplasm; tMN, treatment-related myeloid neoplasm; uMN, treatment-unrelated myeloid neoplasm; WES, whole-exome sequencing.

uMN comparisons. Comparing uMN versus pMN yielded mutation rate differences in PHF6 at 5/48 (10%) versus 15/721 (2%), \(P = 0.006\) and IDH1 at 5/48 (10%) versus 17/720 (2%), \(P = 0.009\) (Figure 3A). Intriguingly, however, for uMN versus tMN, IDH1 mutation rates were 5/48 (10%) versus 1/125 (1%), \(P = 0.007\) (Figure 3B).

Discussion
Our data indicate that specific point mutations associate more with age-related MN than with therapy-related MN, that TP53 mutations are selected for by chemotherapy more than radiotherapy, that EZH2 mutations associate with radiotherapy, that etiologies outside of cancer therapies link other types of cancers to MN via PHF6 and IDH1 mutations, and if the latter holds, that there is an enigmatic lack of IDH1 mutations in tMN.

Mutations in SF3B1, JAK2, IDH1/2, and RAS family genes are dominated by single base changes that activate or alter gene function. An absence of mutations in these genes in tMN supports background processes being more capable of producing specific base changes than cancer therapies. This may reflect greater diversity in types of DNA damaging molecules in background processes, or their persistence and thus heightened odds of overlapping with HSC S-phases, which are rare.

The C>A mutations of JAK2V617F (JAK2G1849T) arise when DNA replicates across unrepaired 8-oxo-guanine (8-oxoG) created by reactive oxygen species (ROS). An absence of JAK2 mutations in tMN implies that the amount of chronic ROS that is produced persistently by electron transport chain leakage, chronic inflammation, or smoking, dwarf the impact and/or the amount of ROS created transiently by chemotherapy or radiotherapy. That myeloproliferative neoplasms (MPN), which are frequently JAK2-mutated, are not known to be associated with radiation is consistent with this interpretation of our finding of higher JAK2 mutation rates in pMN. SRSF2 mutations, many of which are SRSF2P95H also mediated by C>A, were not lower in tMN than in pMN (Table 1). One possible explanation is that in-frame indels also account for many SRSF2 mutations, and these can arise from error prone non-homologous end joining (NHEJ) repair of DNA double strand breaks (DSB) in G0/G1 HSC, so they are expected to be treatment-induced.

Mutations in TET2 and ASXL1 in MN are typically loss-of-function mutations caused by frameshifts that create stop codons that prematurely terminate protein synthesis. Such frameshift mutations are effective at inactivating the gene from a broad range of possible positions in the gene, so the genetic target size of these events is larger than that of specific point mutations such as JAK2V617F. Furthermore, because small deletion frameshifts arise from NHEJ repair of single DSBs, and because NHEJ is the dominant form of DSB repair in the quiescent majority of HSC, as with SRSF2 frameshifts similar frequencies of TET2 and ASXL1 mutations in tMN and pMN (Table 1) are not surprising.
We found that \(TP53\) mutations associate with chemotherapy more than radiotherapy. It has been established that \(TP53\) mutations are not created by chemotherapy and radiotherapy but are rather selected for by them.\(^\text{496}\) We envision mutations that create resistance to cell killing more than growth advantages to establish themselves early in life, when all HSC expand enough to establish clone sizes large enough to avoid clonal extinction via random walks to clone cell numbers of zero. After adulthood is reached, resistance mutation expansions happen opportunistically when neighboring wild-type HSCs are killed by a cytotoxic agent; mutant HSC can then move into the vacated HSC niche and thereby increase in numbers.\(^\text{497}\) \(TP53\) is a resistance gene. \(TP53\) mutations may have been selected for more by chemotherapy than by radiotherapy because chemotherapy is more systemic than radiotherapy, and is thus a pressure on all HSC, and because chemotherapy exposures are more distributed over time than radiotherapy, which is delivered as acute dose fractions. That \(EZH2\) is mutated more in radiotherapy than chemotherapy suggests that either the cell killing resistance it provides is strictly to radiotherapy, not chemotherapy, or that the mutations are not selected for but are instead induced by radiotherapy, and not chemotherapy.

\(EZH2\) exists on chromosome 7q and there is strong evidence that \(EZH2\) loss is a major force behind 7q loss.\(^\text{498}\) In our analyses loss of 7q associated with both chemotherapy-induced tMN and radiotherapy-induced tMN, so while efficient DSB production is a hallmark of radiation, it is likely also a dominant intermediate of chemotherapy and thus not the mechanism by which radiation induces excess \(EZH2\) mutations; further supporting this view, \(EZH2\) mutations of radiotherapy-treated patients did not display deletions/insertion mutations. Another possible mechanism is reactive oxygen species causing 8-oxoG-mediated mutations, namely C>A. In line with this, albeit not with statistical significance, of 5 mutation types arising in \(EZH2\) comparisons of radiotherapy versus no radiotherapy, only C>A arose more in the radiotherapy group than in the no radiotherapy group (eTable 1).

\(PHF6\), \(IDH1\), and \(CUX1\) are mutated in uMN more than in pMN, so germline or environmental exposures that correlate first cancers to uMN may have a connection to these mutations. Mutations in \(PHF6\) and \(CUX1\) are mostly indels, likely via NHEJ DSB repair. If germline mutations in homologous recombination (HR) DSB repair genes caused the correlations between first cancers and uMN, the uMN would then depend more on error-prone NHEJ DSB repair, and may thus be more likely to incur indels in \(PHF6\) and \(CUX1\). Regarding \(IDH1\) mutations, these are C>T that arise by deamination of methylcytosines (mC) to T, or deamination of C to U which also pairs with A. C>T is the most common
Type of mutation. D-2-hydroxyglutarate (D2HG) produced by mutated IDH1 inhibits DSB repair by HR and thus sensitises IDH1 mutant cells to chemotherapy and radiotherapy. This may explain why IDH1 mutation rates are higher in uMN than in tMN: if D2HG sensitises latent subclinical IDH1 mutant clones to cell killing by chemotherapy or radiotherapy administered for a different cancer, chemotherapy, being systemic, could wipe out such a clone, and/or release of IDH1 mutant antigens via chemotherapy or radiotherapy cell killing could stimulate an immune response that keeps the IDH1 mutant clone size subclinical. A genetic explanation for a higher occurrence of IDH1 mutations in uMN versus pMN cases is based on the fact that uMN cases have proven to be able to form at least two primary cancers. For example, a SNP at chromosome locus 8q24.21 is strongly associated with increased risks of gliomas with IDH1 mutations and this involves deregulation of MYC in healthy glial cells, which selects for IDH1 mutations. This or other SNPs may also predispose for other cancers and IDH1-mutated MN, causing our observed higher occurrence of IDH1 mutations in uMN versus pMN.

Relative to background DNA damage, the acuteness of anticancer agent exposures implies that two DSBs are more likely to exist at the same time, as is needed to create dicentrics that achieve the cell-killing intent of cytotoxic therapies. An off-target consequence of this is translocations, large deletions, and complex karyotypes more often found in tMN than pMN. G0/G1 HSC are likely the target cells of MN induction in these cases, as G0/G1 DSB repair depends on error prone NHEJ more than HR.

To the extent that survival is worse in tMN than pMN, our results are consistent with mutations in TP53 and EZH2 predicting worse survival than SF3B1 mutations. An emerging view then is that tMN are a subset of pMN whose mutations are induced or selected for by cytotoxic cancer therapies, and have worse survival. An alternative view, neither supported nor disfavored by our data, is that worse survival is due to comorbidities caused by treatment-associated collateral damage to normal tissue.

Risks of developing MN relative to background rates peak 2-3 years after cancer diagnoses, with higher peaks if the cancer was treated with ionizing radiation, or if it was diagnosed at a younger age (due to lower background rates). Such relative risks are the odds of an MN being a tMN. We substantiated that TP53 mutations and loss of 7q are markers of increased odds of a particular case being a tMN, and suggest that EZH2 mutations further support this if the patient’s prior cancer was treated with radiation. We also found that MN-associated specific point mutations as indeed more likely to be due to aging than due to therapy, and suggest that MN cases with such mutations have lower odds of being tMN cases. Although we do not expect tMN to be treated differently from pMN with similar mutations, improving our understanding of how therapies induce MN may help us adjust cancer therapies so as to minimise subsequent MN risks.