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REVIEW

Therapy-related myeloid neoplasms: pathobiology and clinical characteristics

H Sill[1], W Olipitz[2], A Zebisch[3], E Schulz[1] and A Wölfler[1]

[1]*Department of Internal Medicine, Division of Haematology, Medical University of Graz, Graz, Austria;* [2]*Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA, and* [3]*Systems Biology Ireland, University College Dublin, Dublin, Ireland*

Correspondence
Heinz Sill, Department of Internal Medicine, Division of Haematology, Medical University of Graz, Auenbruggerplatz 38, A-8036 Graz, Austria. E-mail: heinz.sill@medunigraz.at

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Therapy-related myeloid neoplasms (t-MNs) are serious long-term consequences of cytotoxic treatments for an antecedent disorder. t-MNs are observed after ionizing radiation as well as conventional chemotherapy including alkylating agents, topoisomerase-II-inhibitors and antimetabolites. In addition, adjuvant use of recombinant human granulocyte-colony stimulating factor may also increase the risk of t-MNs. There is clinical and biological overlap between t-MNs and high-risk *de novo* myelodysplastic syndromes and acute myeloid leukaemia suggesting similar mechanisms of leukaemogenesis. Human studies and animal models point to a prominent role of genetic susceptibilty in the pathogenesis of t-MNs. Common genetic variants have been identified that modulate t-MN risk, and t-MNs have been observed in some cancer predisposition syndromes. In either case, establishing a leukaemic phenotype requires acquisition of somatic mutations – most likely induced by the cytotoxic treatment. Knowledge of the specific nature of the initiating exposure has allowed the identification of crucial pathogenetic mechanisms and for these to be modelled *in vitro* and *in vivo*. Prognosis of patients with t-MNs is dismal and at present, the only curative approach for the majority of these individuals is haematopoietic stem cell transplantation, which is characterized by high transplant-related mortality rates. Novel transplantation strategies using reduced intensity conditioning regimens as well as novel drugs – demethylating agents and targeted therapies – await clinical testing and may improve outcome. Ultimately, individual assessment of genetic risk factors may translate into tailored therapies and establish a strategy for reducing t-MN incidences without jeopardizing therapeutic success rates for the primary disorders.

Abbreviations

AML, acute myeloid leukaemia; APL, acute promyelocytic leukaemia; CIBMTR, Center for International Bone Marrow Transplantation Research; C/EBPα, CCAAT/enhancer binding protein alpha; CR, complete remission; DSB, double strand break; EBMT, European Group for Blood and Marrow Transplantation; ENU, ethyl-nitrosourea; *FLT3*, *fms*-like tyrosine kinase 3 gene; G-CSF, granulocyte-colony stimulating factor; GWAS, genome-wide association studies; HD, Hodgkin's disease; HSCT, haematopoietic stem cell transplantation; HSPC, haematopoietic stem and progenitor cells; MDS, myelodysplastic syndromes; MGMT, O^6-methylguanine DNA methyltransferase; MMR, mismatch repair; NF1, Neurofibromatosis type 1; *NPM1*, nucleophosmin gene; QTL, quantitative trait loci analysis; RKIP, RAF kinase inhibitor protein; ROS, reactive oxygen species; SEER, Surveillance, Epidemiology, and End Results-Medicare database of the United States; SNP, single nucleotide polymorphism; t-AML, therapy-related acute myeloid leukaemia; t-MDS, therapy-related myelodysplastic syndrome; 6-meTG, 6-thio-methylguanine; t-MNs, therapy-related myeloid neoplasms; TRM, transplantation-related mortality; WHO, World Health Organization

Introduction

Therapy-related myeloid neoplasms (t-MNs) are serious long-term consequences of chemo- and radiotherapy for an antecedent disorder. According to the World Health Organization (WHO) 'Classification of Tumours of Haematopoietic and Lymphoid Tissues', t-MNs comprise therapy-related myelodysplastic syndrome (t-MDS), acute myeloid leukaemia (t-AML) and myelodysplastic/myeloproliferative neoplasm, and constitute a unique clinical syndrome (Vardiman *et al.*, 2009). They are observed after cytotoxic therapies of haematologic malignancies – mainly Hodgkin's disease (HD) and non-Hodgkin's lymphomas – as well as solid neoplasms – most commonly breast, ovarian and prostate cancer. In addition, t-MNs have also been reported in patients receiving immunosuppressive treatment for rheumatologic/autoimmune diseases or solid organ transplantation (Offman *et al.*, 2004; Kwong, 2010). The majority of patients with t-MNs present with myelodysplastic syndrome (MDS) or acute myeloid leukaemia (AML) transformed from MDS after a median latency period of 5–10 years following cytotoxic treatments with alkylating agents, immunosuppressive drugs or radiotherapy. Patients frequently exhibit marked peripheral blood cytopenias and dysplastic features affecting one or more myeloid lineages. Structural aberrations involving chromosomes 5 and 7 or a complex karyotype are commonly detected upon cytogenetic analysis. Twenty to 30% of patients with t-MNs present with overt AML after a latency of 1–5 years. Leukaemic cells predominantly exhibit a monocytic or myelomonocytic phenotype and balanced chromosomal translocations including 11q23 and 21q22 rearrangements or abnormalities such as t(15;17)(q22;q12) and inv(16)(p13q22). A history of previous treatment with topoisomerase-II-inhibitors is common in these individuals. However, as many patients have received multiple lines of treatment including several classes of chemotherapy compounds, both structural and balanced chromosomal aberrations are frequently observed in the leukaemic clone. The WHO has therefore abandoned its former classification into alkylating agent or topoisomerase-II-inhibitor associated therapy-related disease. As a conservative estimate, about 10% of cases of AML and MDS are therapy related (Leone *et al.*, 1999; Mauritzson *et al.*, 2002), but the number of patients with t-MNs is likely to rise due to a steady increase in cancer survivors. Here, we review recent, novel findings on aetiology, susceptibility and treatment of t-MNs in the context of established data that broaden our understanding of the complexity of these disorders. These detailed insights into pathogenetic mechanisms will eventually help to establish a more differentiated clinical approach to successfully treat, but hopefully also prevent, these often fatal consequences of cytotoxic therapies.

Aetiology

Conventional cancer therapeutics preferentially operates by producing extensive DNA damage that in turn inhibits proliferation and activates cell death pathways. Because chemo- and radiation therapies do not target tumour cells exclusively, mutations may also be induced in normal cells. Importantly, if they persist and affect genes controlling growth and differentiation of haematopoietic stem and precursor cells (HSPCs), a neoplastic myeloid clone may arise. In addition, repeated cytotoxic therapies may facilitate the selection of such a clone due to immunosuppression, which is an inevitable side effect of these treatments.

Ionizing radiation

The high incidence of myeloid leukaemias in Nagasaki and Hiroshima atomic bomb survivors firmly established the causal relation between ionizing radiation and haematological malignancies (Little, 1993; Preston *et al.*, 1994; Descatha *et al.*, 2005). Epidemiological studies of patients receiving radiation therapy have since confirmed its leukaemogenic effect (Little, 1993; Travis *et al.*, 2000; Haddy *et al.*, 2006; Le Deley *et al.*, 2007; Ojha *et al.*, 2010). Exposure of cells to ionizing radiation results in the formation of reactive oxygen species (ROS) through radiolysis of water molecules. ROS – most importantly hydroxyl radicals, superoxide radicals and hydrogen peroxide – are highly reactive molecules that can oxidize or deaminate DNA bases and increase the frequency of DNA double strand breaks (DSBs) (Rassool *et al.*, 2007). Radiation photon energy can also directly induce strand breaks by disruption of the sugar phosphate backbone of DNA. DSBs are highly mutagenic, potentially leading to the formation of large scale chromosomal rearrangements that are often found in radiation-induced leukaemias (Philip and Pedersen-Bjergaard, 1988; Rothkamm *et al.*, 2001; Klymenko *et al.*, 2005).

Alkylating agents

Alkylating agents were the first chemotherapeutic compounds to be associated with leukaemia development after successful treatment of solid and haematological cancers (Kyle *et al.*, 1970, 1974; Sypkens Smit and Meyler, 1970; Rosner and Grunwald, 1975; Reimer *et al.*, 1977; Rowley *et al.*, 1981). They comprise a large group of anti-cancer drugs with clinical application across almost all cancer types. Alkylating agents induce DNA damage by transferring alkyl groups – such as $-CH_3$ or $-CH_2-CH_3$ – to oxygen or nitrogen atoms of DNA bases, resulting in highly mutagenic DNA base lesions, such as O^6-methylguanine and N^3-methylcytosine (Saffhill *et al.*, 1985; Horsfall *et al.*, 1990; Shulman, 1993; Drablos *et al.*, 2004). Monofunctional alkylating agents like dacarbazine, procarbazine and temozolomide have one reactive moiety and generally induce base lesions. Alkylated nucleotides are repaired by the nucleotide and base excision repair systems. The O^6-methylguanine lesion is predominantly repaired by O^6-methylguanine DNA methyltransferase (MGMT) transferring the methyl lesion to a cysteine residue within its active site (Margison and Santibanez-Koref, 2002; Drablos *et al.*, 2004; Allan and Travis, 2005). However, MGMT expression is highly variable in human (tumour) cells and the persistence of O^6-methylguanine can result in secondarily formed DNA DSBs (Gerson *et al.*, 1996; Kaina *et al.*, 2007). In contrast to other lesions, O^6-methylguanine efficiently causes mispairing during DNA replication. Although these mispaired bases elicit a DNA-mismatch repair (MMR) response, the methylated base cannot be cleaved by MMR proteins, eventually leading to cytotoxicity and mutagenicity (Allan

and Travis, 2005). Accordingly, experimental systems modifying MGMT activity have revealed that O^6-methylguanine is a major mutagenic, carcinogenic, recombinogenic but also cytotoxic lesion induced by alkylating agents (Kaina *et al.*, 2007). In contrast to monofunctional alkylators, bifunctional alkylating agents have two reactive sites and include agents such as melphalan, cyclophosphamide and chlorambucil. Thus, in addition to DNA base lesions, they therefore can form intra- and interstrand crosslinks by attacking two bases within the same or on opposing DNA strands respectively. During replication, interstrand crosslinks stall replication forks, which can result in the formation of DNA DSBs. If misrepaired or left unrepaired, DNA DSBs can give rise to translocations, inversions, insertions and loss of heterozygosity (Richardson and Jasin, 2000; Helleday *et al.*, 2008).

Topoisomerase inhibitors

In addition to alkylating agents, DNA topoisomerase inhibitors were identified as inducing a distinct form of t-MNs (Pedersen-Bjergaard *et al.*, 1991). While alkylating agents associated with t-MNs are characterized by a complex karyotype often featuring partial or complete loss of chromosomes 5 and/or 7, exposure to topoisomerase inhibitors leads to the development of leukaemias with balanced translocations involving *MLL* at 11q23, *RUNX1* at 21q22 and *RARA* at 17q21 (Pedersen-Bjergaard *et al.*, 1995; Dissing *et al.*, 1998; Smith *et al.*, 2003). DNA topoisomerases are critical enzymes responsible for unknotting and relaxing supercoiled DNA, thus allowing DNA replication to occur. To relax supercoiled DNA, toposiomerases bind covalently to the DNA strand and create transient single (type I topoisomerases) and DSBs (type II topoisomerases). These DNA strand breaks are readily religated after topoisomerases are released from the DNA (Nitiss, 2009). As these ubiquitous enzymes are essential to cell survival, DNA topoisomerases have become a valuable target for several cytostatic drugs, such as epipodophyllotoxins and anthracyclines. Topoisomerase inhibitors block the release of topoisomerases from cleaved DNA, preventing religation of the DNA strands (Allan and Travis, 2005). Thus, topoisomerase inhibitors lead to the generation of permanent DNA DSBs that trigger DSB-induced apoptosis. However, persistent DNA DSBs are also highly mutagenic and can result in chromosomal deletions, insertions, inversions and translocations, all of which are characteristic of the leukaemic cell clone in t-MNs. The exact molecular effects of these inhibitors on the acquisition of chromosomal aberrations and the development of this t-MN subtype have recently been reviewed in detail (Joannides and Grimwade, 2010).

There is a recent report of a novel association between topoisomerase inhibition and risk of secondary myeloid neoplasms (Tebbi *et al.*, 2007). Dexrazoxane – a bisdioxopiperazine iron chelator used to reduce cardiopulmonary toxicity in patients treated with anthracyclines – also interferes with topoisomerase II in its dimerized state by bridging and stabilizing the ATPase region. In a randomized phase III study in paediatric patients treated with chemo- and radiotherapy for HD, dexrazoxane was associated with a cumulative incidence of MDS/AML of 2.5% ± 1.0% as compared with 0.85% ± 0.6% for the non-dexrazoxane group ($P = 0.16$). This trend towards an increased risk of secondary neoplasms associated with dexrazoxane was subsequently confirmed in patients with childhood acute lymphoblastic leukaemia (Salzer *et al.*, 2010).

Antimetabolites

Antimetabolites, such as fludarabine, azathioprine and 6-thioguanine, are yet another group of cytostatic drugs causally involved in the development of t-MNs (Smith *et al.*, 2003; Offman *et al.*, 2004; Leleu *et al.*, 2009). Antimetabolites are incorporated into DNA, thereby interfering with replication and leading to cell cycle arrest and apoptosis. Azathioprine is widely used as an immunosuppressant in patients with autoimmune disorders or recipients of solid organ transplants. Similar to 6-mercaptopurine, it is metabolized to 6-thioguanine, a guanine nucleotide analogue, which is incorporated into DNA during replication. Once placed in the newly synthesized DNA strand, 6-thioguanine is prone to methylation and formation of the highly mutagenic base lesion 6-thio-methylguanine (6-meTG) that closely resembles the O^6-methylguanine lesion induced by alkylating agents. Cell cycle arrest and cell death after azathioprine treatment are triggered by the DNA MMR machinery (Waters and Swann, 1997; McLeod *et al.*, 2000). However, MMR-deficient cells can tolerate 6-meTG, potentially forming a leukaemic clone (Offman *et al.*, 2004; Treon *et al.*, 2009). In line with the cytogenetic aberrations found with alkylating agents, patients with t-MNs after azathioprine treatment frequently harbour partial or complete loss of chromosomes 5 and 7. Fludarabine and other nucleoside antagonists are increasingly used in combination with alkylating agents in patients with indolent lymphoma, exposing these individuals to a substantial risk of developing t-MNs (Morrison *et al.*, 2002; Leleu *et al.*, 2009; Treon *et al.*, 2009).

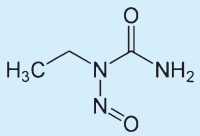
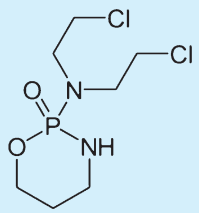
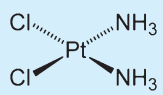
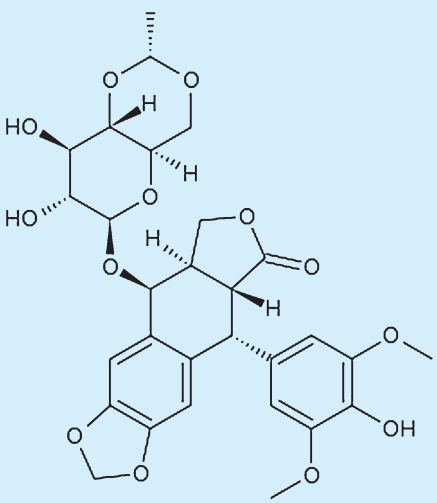
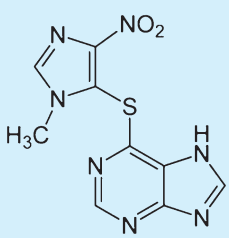
As outlined previously and in other reviews (Allan and Travis, 2005; Joannides and Grimwade, 2010; Leone *et al.*, 2010), the molecular events induced by the interaction of different cytotoxic regimens with the DNA and their potential role in the pathogenesis of t-MNs are being increasingly delineated (Table 1). In clinical practice, however, t-MN patients most often present after treatment with complex chemotherapeutic schedules sometimes combined with radiation therapy, making it difficult to identify the causative agent in any particular case. While the combination of chemo- and radiotherapy may further increase the risk of t-MNs, especially when regimens including total body irradiation are applied, the influence of a cumulative dose of chemotherapeutic drugs is still a matter of debate (Leone *et al.*, 1999; Travis *et al.*, 2000; Le Deley *et al.*, 2007; Lyman *et al.*, 2010). Furthermore, the risk of developing t-MNs may also be modulated by other drugs, such as haematopoietic growth factors.

Granulocyte-colony stimulating factor

Since its clinical availability in the early 1990s, recombinant granulocyte-colony stimulating factor (G-CSF), an essential cytokine for the production of neutrophilic granulocytes, has been widely used to reduce the severity and the duration of neutropoenia, the risk of febrile neutropoenia and infection-related mortality in cancer patients receiving chemotherapy (Clark *et al.*, 2005; Kuderer *et al.*, 2007). G-CSF also enables the delivery of dose-intense and dose-escalating

Table 1

Main chemotherapeutic classes associated with therapy-related myeloid neoplasms, their mode of action and repair mechanisms involved

	Structure	Toxic DNA lesions	Major repair mechanisms
Monofunctional alkylators			
Ethyl-nitrosourea Procarbazine Dacarbazine Temozolomide		Base damage Replication lesions Bulky adducts	MGMT Base excision repair Nucleotide excision repair Homologous recombination Fanconi anaemia repair pathway
Bifunctional alkylators			
Cyclophosphamide Ifosphamide Mitomycin C Melphalan Chlorambucil		Base damage DNA inter-/intrastrand crosslinks Replication lesions Bulky adducts Double strand breaks	MGMT Base excision repair Nucleotide excision repair Homologous recombination Fanconi anaemia repair pathway
Cisplatin Carboplatin			
Topoisomerase inhibitors			
Etoposide Doxorubicin Daunorubicin Epirubicin Mitoxantrone Camptothecin		Double strand breaks Single strand breaks Replication lesions	Non-homologous end joining Homologous recombination Fanconi anaemia repair pathway
Antimetabolites			
Azathioprine Fludarabine Cladribine 5-Fluorouracil		Base damage Replication lesions ?	Base excision repair ?

The structural formulas are given for those drugs stated first within either class except for cisplatin. Platinum compounds are correctly classified as alkylating-like agents as they have no alkyl group. They nevertheless act by forming DNA interstrand crosslinks. For references, see main text of the manuscript.
MGMT, O6-methylguanine DNA methyltransferase.

chemotherapy regimens that could not otherwise be administered safely, thereby increasing the response and survival rates for distinct cancer entities. However, several recent reports have expressed concerns about an increased risk of developing t-MNs in patients receiving G-CSF during chemotherapy (Relling *et al.*, 2003; Hershman *et al.*, 2007; Le Deley *et al.*, 2007).

G-CSF stimulates the proliferation of granulocytic progenitors and promotes their differentiation into mature neutrophils (Beekman and Touw, 2010). It also causes premature release of neutrophils from the bone marrow and enhances their capacity for phagocytosis, ROS generation and bacterial cell killing. In addition, G-CSF induces the release of proteases in activated bone marrow neutrophils, facilitating the

mobilization of HSPCs into the peripheral blood. Accordingly, G-CSF is administered for the collection of HSPCs in both autologous and allogeneic donor settings (Levesque and Winkler, 2008; Trumpp *et al.*, 2010). Two mechanisms have been implicated in the G-CSF-mediated promotion of t-MNs. First, G-CSF-induced production and release of ROS by bone marrow neutrophils may result in increased DNA damage and mutation rates in HSPC (Touw and Bontenbal, 2007). Second, repeated application of G-CSF results in a continuous egress of these cells from their protective bone marrow niche, which may render them more susceptible to genotoxic stress (Trumpp *et al.*, 2010).

In a recent meta-analysis, Lyman *et al.* (2010) evaluated the risk of t-MNs in patients undergoing chemotherapy randomly assigned to receive G-CSF. They identified 23 eligible clinical trials with more than 6000 patients in each group and reported an absolute risk increase of 0.43%. All-cause mortality was lower in the group of patients randomized to G-CSF, with a decrease in the absolute risk for death of 3.4%. This reduction was attributed to a lower cancer-related mortality due to a more dose-dense and dose-escalated application schedule of chemotherapy regimens. However, no significant association between the relative dose-intensity of the delivered chemotherapy and the risk for t-MNs was observed. Despite the clear results of this meta-analysis, some limitations have to be taken into account: first, the development of t-MNs was not a primary endpoint in any of the included trials and – as also mentioned by the authors – their true incidence might have been under-reported in the studies analysed. Second, in many included trials, G-CSF could also be administered to patients initially randomized to the control group in subsequent chemotherapy cycles, but the absolute G-CSF dose received by each patient was not reported in any trial. Thus, a dose-dependent effect of G-CSF on the risk of developing myeloid neoplasms, as has been noted for patients with severe congenital neutropoenia (Rosenberg *et al.*, 2006), might have been missed. However, despite these limitations, this meta-analysis clearly indicates that the administration of G-CSF for the treatment of chemotherapy-related neutropoenia and its complications benefits a substantial proportion of patients and outweighs the increased t-MN risk.

Therapy-related versus second primary versus *de novo* myeloid neoplasms

The WHO classified t-MNs as a late complication of cytotoxic chemo- and/or radiotherapy (Vardiman *et al.*, 2009). In a population-based study using data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare database of the United States, patients with non-metastatic breast cancer older than 65 years were analysed for the occurrence of AML (Patt *et al.*, 2007). The absolute AML risk at 10 years was 1.8% for more than 10 000 women who received adjuvant chemotherapy and 1.2% for almost 55 500 women who did not. Adjuvant chemotherapy accounted for an increase in AML risk of 53%. Based on these data, a proportion of AML cases in the adjuvant chemotherapy group may not be attributable to previous cytotoxic therapies but developed as a second primary malignancy. Another SEER analysis reporting AML incidences following various treatment modalities for localized prostate cancer confirmed the increased risk for AML after cytotoxic treatments – in these cases external beam radiotherapy (Ojha *et al.*, 2010).

According to the WHO classification, the distinction of t-MNs from *de novo* myeloid malignancies is solely based on a patient's history but not on specific molecular, cytogenetic or cellular markers. Nevertheless, several studies have been conducted aiming to define cases of MDS/AML occurring after a primary malignancy as therapy related. In a GIMEMA study of 179 patients with secondary AML, a higher rate of chromosome 5 or 7 abnormalities was shown in the group of therapy-related leukaemias (Pagana *et al.*, 2001). Consistent with the GIMEMA data, chromosome 5 and 7 abnormalities were significantly associated with multiple lines of previous therapies in patients with MDS/AML evolving from polycythaemia vera. In contrast, trisomy 8 and 9 were the most frequent finding in those treated with phlebotomy only (Swolin *et al.*, 2008). However, no cytogenetic aberration has yet been proven to be specific for t-MNs.

Myeloid neoplasms associated with occupational or environmental exposure to leukaemogenic agents show striking similarities to t-MNs. For example, the relative risk of MDS and AML following occupational benzene exposure is increased as compared with the general population. In addition, CD34+ HSPCs incubated with benzene metabolites show a propensity to develop chromosome 5 and 7 abnormalities (Stillman *et al.*, 2000; Descatha *et al.*, 2005). Recent data also point towards an increased cancer risk due to low dose irradiation following diagnostic X-ray or computed tomography examinations (Sigurdson *et al.*, 2008; Mullenders *et al.*, 2009; Smith-Bindman *et al.*, 2009).

Interestingly, high-risk *de novo* MDS/AML cases also share biological and clinical features with t-MNs. These include chromosomal 5 and/or 7 abnormalities as well as low response rates to intensive chemotherapies and haematopoietic stem cell transplantation (HSCT). As the proportion of patients with high-risk *de novo* MDS/AML increases with age, it is hypothesized that chronic environmental stress may contribute to MDS/AML development (Appelbaum *et al.*, 2006). Thus, high-risk *de novo* MDS/AML leukaemogenesis resembles t-MN development except that no specific cytotoxic agent(s) could be identified in the patient's history (Figure 1). Studying t-MNs may allow a better understanding of high-risk *de novo* MDS/AML, potentially leading to strategies of improved treatment and prevention for both entities.

Despite the lack of specific cytogenetic and cellular t-MN markers and the apparent clinical overlap with high-risk *de novo* MDS/AML, some evidence has been obtained for distinct molecular characteristics of t-MNs. In the majority of cases that developed acute promyelocytic leukaemia (APL) after treatment with the topoisomerase-II-inhibitor mitoxantrone but not in *de novo* APL, a tight clustering of breakpoints within an 8-bp region in *PML* intron 6 was observed (Mistry *et al.*, 2005). *In vitro* studies with a double strand DNA substrate, homologous to the PML translocation site, showed marked cleavage at the 8-bp hot spot after incubation with mitoxantrone and etoposide. Similar results of preferential sites of DNA damage induced by topoisomerase-II targeted drugs were obtained for APL cases following mitoxantrone

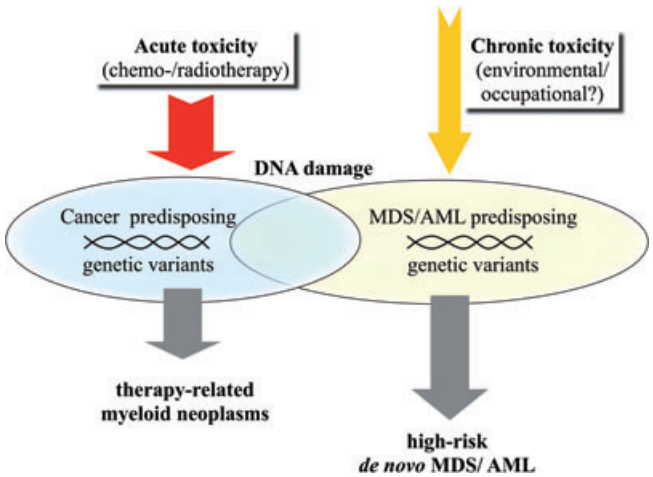


Figure 1

Similarities between therapy-related and high-risk *de novo* myeloid leukaemogenesis. In therapy-related myeloid neoplasms high doses of mutagenic chemo-/radiotherapy impact on the DNA of haematopoietic stem and precursor cells. In contrast, chronic exposure to low doses of occupational/environmental agents over extended periods of time may be operational in the development of high-risk *de novo* MDS/AML. Genetic variants conferring predisposition to the primary malignacy may also be of relevance for therapy-related leukaemogenesis and account for subtle biologic differences between t-MNs and high-risk *de novo* MDS/AML. MDS, myelodysplastic syndrome (MDS); AML, acute myeloid leukaemia.

treatment for multiple sclerosis and epirubicin for breast cancer (Hasan *et al.*, 2008; Mays *et al.*, 2010). The authors suggest a mechanism whereby topoisomerase-II inhibitor-induced DNA DSBs are formed in susceptible regions of the genome that are erroneously repaired by non-homologous end-joining. Thus, chromosomal translocations may arise and result in the development of a malignant clone.

Genetic susceptibility

The fact that a small but constant proportion of individuals receiving identical cytotoxic regimens develop t-MNs pinpoints a contribution of genetic risk factors in the pathogenesis of these disorders. This concept received further support from studies that demonstrated a family history of cancer in the majority of patients with t-MNs (Ben-Yehuda *et al.*, 1996; Pagana *et al.*, 2001). Genetic predisposition to t-MNs is, however, regarded a complex trait determined by multiple pathogenetic variants and their interaction with specific exogenous toxicities. Preliminary evidence for susceptibility factors comes from both animal models and human studies.

Animal models

Mouse models have become an indispensable tool for studying genetic predisposition to therapy-related myeloid leukaemogenesis. To test the concept that heritability contributes to alkylator-induced oncogenesis, Graubert *et al.* treated different inbred mouse strains with ethyl-nitrosourea (ENU), a prototypical alkylating agent (Fenske *et al.*, 2006). Indeed, a high interstrain variability of therapy-induced solid and haematologic malignancies was observed and a particular strain – SWR/J – identified with pronounced susceptibility to t-MNs. To identify alleles of the SWR/J strain that mediate ENU-induced leukaemia susceptibility, quantitative trait loci (QTL) analysis of F2 intercrosses from strains susceptible or resistant to alkylator-induced leukaemia was performed. A set of QTLs and candidate quantitative trait genes implicated in DNA damage response and repair as well as apoptosis was identified (Fenske *et al.*, 2006).

A commonly used approach to model genetic predisposition to both *de novo* and therapy-induced myeloid neoplasms in mice is to introduce recurrent genetic aberrations found in human AML into the murine haematopoietic system and determine the frequency of leukaemias that arise spontaneously or following exposure to cytotoxic agents. Deletion of the long arm of chromosome 5 is found in about 10% of patients with *de novo* MDS and AML and 40% with t-MNs (Thirman and Larson, 1996). The transcription factor Early Growth Receptor 1 (*EGR1*) has been identified as a candidate tumour suppressor gene located in the commonly deleted region of chromosome 5 (Lai *et al.*, 2001). EGR1 regulates genes like *TP53*, *PTEN*, *CDKN1A* and *TGF-β*, thereby controlling cell proliferation and apoptotic pathways (Baron *et al.*, 2006). Egr1 knockout and haploinsufficient mice display only minor haematopoietic abnormalities. However, following treatment with the alkylating agent ENU, 40% of $Egr1^{-/-}$ and 33% of $Egr1^{+/-}$ mice develop a myeloproliferative disorder, as compared with only 13% of wild-type mice (Joslin *et al.*, 2007). Thus, Egr1 deficiency predisposes animals to myeloproliferative diseases in the presence of additional mutations induced by alkylating agents. Importantly, haploinsufficiency of Egr1 is as potent in predisposing to therapy-induced leukaemia as complete loss of Egrl. *RUNX1/ETO* (also known as *AML1/ETO*) is a fusion gene resulting from the chromosomal translocation t(8;21)(q22;q22) that is present in about 12–15% of AML cases. *RUNX1/ETO* inhibits the transcription factor CCAAT/enhancer binding protein alpha (C/EBPα), resulting in a block of granulocytic differentiation at the myeloid progenitor cell level (Westendorf *et al.*, 1998; Wölfler *et al.*, 2010). Introducing *RUNX1/ETO* into murine haematopoietic stem cells is not sufficient to induce AML development, even after long latency periods of up to 24 months (de Guzman *et al.*, 2002; Higuchi *et al.*, 2002). Similar to mice with Egr1 deficiency, the induction of cooperating mutations through treatment with ENU results in the development of AML in 31% of mice with the conditional *RUNX1/ETO* oncogene while no leukaemia was observed in any of the ENU treated wild-type mice. This result demonstrates that *RUNX1/ETO* is not able to induce leukaemogenesis by itself, but predisposes to the development of murine myeloid leukaemias following alkylation treatment. Another common genetic abnormality in human AML is the *CBFβ-MYH11* fusion gene as a consequence of a chromosome 16 inversion/translocation – inv(16)(p13;q22)/t(16;16)(p13;q22). In this aberration, the function of the transcription factor Cbfβ is impaired, resulting in a differentiation stop of myeloid cells (Castilla *et al.*, 1996). When expressed in mice, *CBFβ/MYH11* – similar to *RUNX1/ETO* – does result in leukaemia formation only after treatment with ENU (Castilla *et al.*, 1999).

Exploiting inherited diseases associated with increased leukaemia risk is another approach to investigating predisposition to t-MNs in animal models. Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder showing predisposition to benign neurofibromas and malignant peripheral nerve sheet tumours as well as juvenile myelomonocytic leukaemia (Stiller *et al.*, 1994; Lauchle *et al.*, 2006). NF1 is caused by heterozygous germline mutations in the *NF1* gene that encodes neurofibromin, a negative regulator of the RAS-MAPK pathway. To investigate the role of *NF1* in murine therapy-induced leukaemogenesis, Shannon and colleagues treated wild-type and heterozygous *Nf1* knockout mice with the alkylator cyclophosphamide and the topoisomerase-II-inhibitor etoposide (Mahgoub *et al.*, 1999). A myeloproliferative disorder was observed in both cohorts of animals but a significantly higher incidence of AML with shorter latency was detected in *Nf1*+/– mice that received cyclophosphamide. Genetic analysis revealed loss of the wild-type *Nf1* allele in a substantial proportion of cyclophosphamide-induced leukaemias, indicating that alkylation treatment is associated with the inactivation of specific target suppressor genes, such as *Nf1*. 129/SV mice were more susceptible to cyclophosphamide-induced LOH at *Nf1* than the 129/SV × C57Bl/6 intercross supporting again the concept that the overall genetic background is an important modulator of t-MN susceptibility.

Human studies

A number of case-control studies have focused on constitutional genetic variants that modulate the risk of developing t-MNs. Initial research investigated polymorphisms within candidate genes of pathways involved in the metabolism of genotoxic agents including cytochrome p450, glutathione S-transferase and NAD(P)H : quinone oxidoreductase genes. An area of extensive investigations pertained to altered DNA repair as the DNA constitutes the major target for most cytotoxic treatments. Indeed, several constitutional variants in genes involved in major human DNA repair pathways have been reported in patients with t-MNs. Previous reviews have summarized and discussed the results of these studies (Allan and Rabkin, 2005; Seedhouse and Russell, 2007; Leone *et al.*, 2010). Although these approaches produced novel insights into susceptibility to t-MNs, several objections have been raised. Statistical power is often weak due to small sample sizes and a heterogeneous disease population as patients with different types of t-MNs are included. In addition, the choice of a proper control group for case-control studies of therapy-related neoplasms is still being discussed. Although cases of *de novo* MDS/AML are generally regarded as inappropriate controls, there is still debate whether healthy individuals or patients with the same primary disorders who have not developed t-MNs are more appropriate (Seedhouse and Russell, 2007). Moreover, the functional consequences of some genetic variants associated with predisposition to t-MNs have not been validated or showed divergent results in cellular assays or mouse models.

Recently, epistasis – the interactive effect of different single nucleotide polymorphisms – was studied in the context of t-MNs (Ellis *et al.*, 2008). Individuals with certain allelic variants within the *MDM2* and *TP53* genes – both involved in the TP53 DNA damage response pathway – were at significantly increased risk for chemotherapy-related AML; however, no effect was seen with either polymorphism alone. Over the past few years, genome-wide association studies (GWAS) have substantially increased our knowledge of susceptibility factors for several complex traits and diseases (Pearson and Manolio, 2008; Visscher *et al.*, 2008; Manolio *et al.*, 2009; Sebastiani *et al.*, 2009; Hartman *et al.*, 2010; Ioannidis *et al.*, 2010; Speicher *et al.*, 2010). This approach enables an objective assessment of the entire genome, is not hypothesis driven regarding genetic association with a particular disease and therefore represents an important development beyond candidate gene studies. However, the odds ratios of susceptibility alleles identified by GWAS for common malignancies like breast and colon cancer are in the range of 1.2 to 1.5, implying a modest contribution towards heritability of these disorders. Under the assumption of a greater effect size of risk alleles, a GWAS in 80 patients with t-MNs and matched controls was performed (Knight *et al.*, 2009). Using a 10 K array, 15 single nucleotide polymorphisms (SNPs) at a significance threshold of $P < 0.001$ were identified. No evidence for recurrent constitutional copy number alterations was found. Three of the SNPs were associated with abnormalities of chromosomes 5 and/ or 7, and a number of genes are in linkage disequilibrium with these SNPs. Unexpectedly, no associations with previously described SNPs of drug detoxification and DNA repair genes have been reported.

The development of t-MNs has also been observed in individuals with hereditary cancer predisposition syndromes after successful treatment of their primary malignancies. The Li-Fraumeni and the Li-Fraumeni-like syndromes are autosomal dominant cancer predisposition syndromes with incomplete penetrance associated with germline mutations of the *TP53* gene. They are characterized by a variety of early onset malignancies including sarcomas, breast and adrenocortical carcinomas, and brain tumours (Bougeard *et al.*, 2008; Gonzalez *et al.*, 2009). In their initial report, Li *et al.* (1988) described six individuals out of 24 kindreds who developed seven secondary malignancies within the radiotherapy field, suggesting that *TP53* germline mutations confer an increased risk of therapy-related neoplasms. Subsequent reports described the occurrence of t-MNs in singular cases with deleterious *TP53* germ-line mutations but no consistent association has been reported to date (Dockhorn-Dworniczak *et al.*, 1996; Felix *et al.*, 1996; Hisada *et al.*, 1998; Kuribayashi *et al.*, 2005; Talwalkar *et al.*, 2010).

NF1, Costello-, LEOPARD- and Noonan syndrome are developmental disorders with increased cancer and leukaemia risk that are associated with germline mutations in genes of the RAS-MAPK pathway (Lauchle *et al.*, 2006; Zebisch *et al.*, 2007; Denayer *et al.*, 2008). Single patients with NF1 who developed t-MNs following chemotherapy for a variety of preceding neoplasms have been described (Perilongo *et al.*, 1993; Papageorgio *et al.*, 1999). A survey of the tumour registry of the Children's Hospital of Philadelphia, USA, produced an incidence of secondary malignancies of 10% among 64 NF1 patients treated with cytotoxic therapies including irradiation (Maris *et al.*, 1997). Bone marrow analysed from four NF1 cases with secondary MDS and monosomy 7 did not show loss of heterozygosity at the *NF1* locus or activating RAS mutations. However, Nf1 haploinsufficiency may be able to induce myeloid leukaemias when functionally

cooperating with other oncogenic events as has been demonstrated in a mouse model of Nf1 haploinsuffiency and deficiency of the interferon consensus sequence binding protein (Koenigsmann *et al.*, 2009). Noonan-syndrome is an autosomal dominant developmental disorder showing germline mutations in the *PTPN11*, *SOS1*, *KRAS* or *RAF1* gene (Tartaglia *et al.*, 2001; Pandit *et al.*, 2007; Razzaque *et al.*, 2007; Roberts *et al.*, 2007). t-AML has been reported in this syndrome along with myeloproliferative neoplasms and juvenile myelomonocytic leukaemia (Chantrain *et al.*, 2007). Recently, we described *RAF1* germline mutations in non-syndromic patients with t-AML (Zebisch *et al.*, 2006). These mutations were located in the highly conserved protein kinase domain and exhibited transforming and anti-apoptotic properties *in vitro*. Interestingly, the MAPK-ERK pathway was constitutively activated in both the primary tumours and the t-AMLs, implicating a contribution of *RAF1* mutations towards the pathogenesis of either disease. However, the activation of ERK was restricted to neoplastic tissues, suggesting the requirement for cooperating mutations. We identified loss of the RAF kinase inhibitor protein (RKIP) as a somatic, leukaemia-specific event, and demonstrated that RKIP significantly influences the transformation potential of mutant Raf1 *in vitro* (Zebisch *et al.*, 2009).

A novel concept addresses epigenetic modification as an important factor in conferring disease susceptibility (Petronis, 2010). In somatic cells, these changes occur either stochastically or as a consequence of environmental influences and are transmitted to daughter cells, albeit with less fidelity than DNA sequence variants. Epigenetic variation also occurs in germ cells. Although the zygote is epigenetically reprogrammed after fertilization, growing evidence points towards an – at least partial – retention of DNA methylation profiles. Epigenetic modification affecting critical loci of haematopoietic stem and precursor cells might add to an increased risk of t-MNs following cytotoxic treatment. Indeed, extensive aberrant DNA methylation of neoplastic cells of patients with *de novo* and therapy-related MDS/AML has been reported recently (Jiang *et al.*, 2009; Voso *et al.*, 2010), but its role in conferring susceptibility to these neoplasms remains to be determined.

Prognosis and treatment

Data on prognostic parameters and treatment outcome are limited in patients with t-MNs and often based on retrospective analyses. Even though individuals with t-MNs are infrequently enrolled in clinical trials, it is well recognized that their prognosis is dismal (Mauritzson *et al.*, 2002; Schoch *et al.*, 2004). Large, unselected cohort studies revealed a median survival of 8–10 months and a 5 year overall survival of less than 10% (Smith *et al.*, 2003; Rund *et al.*, 2005). t-MDS exhibits a higher transformation rate to leukaemia as compared with *de novo* MDS (Smith *et al.*, 2003; Singh *et al.*, 2007). Based on the data of the German AML Cooperative Group, the influence of chromosomal aberrations on outcome parameters was compared between *de novo* and t-AML cases, both treated uniformly within prospective protocols (Kern *et al.*, 2004; Schoch *et al.*, 2004). Karyotype was an independent prognostic parameter in *de novo* and t-AML patients, but unfavourable karyotypes were more frequent in the t-AML group. Furthermore, disease outcome was inferior in t-AML as compared with *de novo* AML patients within all cytogenetic risk groups. These data establish t-AML as an independent adverse prognostic factor.

In addition to cytogenetic data, somatic gene mutations are increasingly recognized as important prognostic markers in AML patients. Assessment of frame shift mutations of the nucleophosmin gene (*NPM1*), internal tandem duplications of the *fms*-like tyrosine kinase 3 gene (*FLT3*) and double mutations in the C/EBPα is now recommended as part of the clinical workup of patients with cytogenetically normal AML and increasingly incorporated into therapeutic decision algorithms (Dohner *et al.*, 2010). Both *FLT3* internal tandem duplications and *NPM1* mutations have also been described in patients with t-MNs (Christiansen and Pedersen-Bjergaard, 2001; Au *et al.*, 2004; Side *et al.*, 2004; Andersen *et al.*, 2008). However, these mutations are associated with a normal karyotype and therefore significantly less prevalent in a t-MN cohort, making the determination of their prognostic value difficult.

Established therapeutic approaches for patients with t-MNs include supportive care, chemotherapy and allogeneic HSCT. Early clinical studies focused on the role of intensive chemotherapy in this patient cohort. Although complete remissions (CR) have been achieved, remission rates are lower and remission duration is shorter than in *de novo* MDS/AML (Larson *et al.*, 1988; Hoyle *et al.*, 1989; Kantarjian *et al.*, 1993; Takeyama *et al.*, 2000). In a retrospective analysis of 122 patients with t-AML treated at the MD Anderson Cancer Center with cytosine-arabinoside, there was a CR rate of 37%. In the same report, the results of 13 different studies including a total of 496 patients with t-AML were summarized, revealing an overall CR rate of 27% (Kantarjian *et al.*, 1993). However, the University of Chicago group recently reported an overall remission rate of 82% with high-dose cytarabine/mitoxantrone in previously untreated patients with t-MNs (Godley *et al.*, 2010). For patients who developed t-MNs with favourable karyotypes – APL with t(15;17) and core-binding factor leukaemias with either t(8;21) or inv16/t(16;16) – treatment is recommended with high-dose chemotherapy in accordance with guidelines for their *de novo* counterparts. A comparably good treatment outcome for therapy-related APL patients could be demonstrated in two large studies from Italy and France. Remission rates were 97 and 80%, respectively, with regimens containing all-trans retinoic acid and survival was reported as 65% at 4 years in the Italian study and 59% at 8 years in the French study (Pagana *et al.*, 2001; Beaumont *et al.*, 2003). However, this concept has recently been challenged for therapy-related core-factor binding leukaemias. A significantly inferior event-free and overall survival was reported for t-AML patients with t(8;21) and inv(16) when compared with their *de novo* counterparts, raising the question of the optimal treatment approach for this patient cohort (Schnittger *et al.*, 2007; Borthakur *et al.*, 2009; Gustafson *et al.*, 2009). Further studies will have to determine whether different cooperating mutations account for this phenomenon.

For the majority of patients with t-MNs, allogeneic HSCT offers the only chance of long-time disease-free survival. Early studies reporting retrospective data on myeloablative HSCT

from related and unrelated donors revealed disease-free survival rates of up to 30% at 5 years. However, transplantation-related mortality defined as death in CR of the neoplastic disease was as high as 58% (Yakoub-Agha *et al.*, 2000; Witherspoon *et al.*, 2001). Surprisingly, even in paediatric patients with t-MNs, low survival and high transplantation-related mortality rates following HSCT were observed, raising the question of toxicity issues in patients who have been heavily pretreated for a primary disorder (Woodard *et al.*, 2006; Aguilera *et al.*, 2009). In subsequent studies, outcome parameters of HSCT were compared with *de novo* MDS/AML patients. In an update of the Seattle cohort, 251 patients with secondary MDS/AML following antecedent haematologic disorders or cytotoxic therapies and 339 patients with *de novo* MDS/AML were analysed (Chang *et al.*, 2007). Relapse-free survival rates were dependent on the conditioning regimen and between 20 and 47% at 5 years. Again, there was a high a non-relapse mortality of up to 54%. When adjusted for risk factors other than disease aetiology, there were no significant differences between the secondary and *de novo* disease cohorts. These data indicate that HSCT might compensate for the differences observed between conventionally treated secondary and *de novo* MDS/AML patients possibly due to a graft-versus-leukaemia effect. The Dana-Farber group focused on the impact of karyotypic aberrations as an important outcome parameter for patients with MDS/AML undergoing HSCT (Armand *et al.*, 2007) and developed a novel cytogenetic classification scheme that predicted outcome more accurately than established ones for both *de novo* as well as therapy-related disease. These data emphasize the close biological relationship between high-risk MDS/AML irrespective of disease aetiology. Recently, the European Group for Blood and Marrow Transplantation (EBMT) and the Center for International Bone Marrow Transplantation Research (CIBMTR) published risk scores based on the registry data of large patient cohorts with t-MNs undergoing HSCT (Kroger *et al.*, 2009; Litzow *et al.*, 2010). These risk scores were highly predictable of transplantation outcome and included age, cytogenetics, disease status at transplantation and donor characteristics. Patients with a favourable risk score showed an overall survival of 63% in the EBMT and 50% in the CIBMTR registry respectively. However, the majority of patients with t-MNs exhibited several risk factors that were associated with inferior outcome. Preliminary data indicated that non-myeloablative HSCT was not associated with a more favourable outcome, but this needs further evaluation in prospective clinical trials. In those t-MN patients who lack a suitable stem cell donor, autologous stem cell transplantation should be considered as an alternative option (Kroger *et al.*, 2006).

Novel drugs have recently been introduced to treat MDS/AML. Lenalidomide, an immunomodulatory agent, is now approved by the US Food and Drug Administration for patients with transfusion-dependent primary, lower-risk MDS associated with interstitial deletion of the long arm of chromosome 5. However, experience in this cohort is limited to small case series because neither initial nor subsequent studies included patients with t-MDS (List *et al.*, 2006; Melchert *et al.*, 2007; Raza *et al.*, 2008). In randomized phase III clinical trials, azacytidine, a DNA methyltransferase inhibitor, has significantly improved overall survival in patients with *de novo* high-risk MDS/AML with low bone marrow blast counts (Fenaux *et al.*, 2009; 2010). Again, data for patients with t-MNs are lacking, revealing the difficulty of evaluating the efficacy of novel drugs in these orphan diseases.

Concluding remarks

In the 1970s, t-MNs were recognized as severe long-term consequences of cytotoxic therapies for a primary disorder. Since then, numerous studies have outlined clinical and biological features of t-MNs leading to their classification as a separate disease entity by the WHO. The prospects of patients with t-MNs, nonetheless, remain dismal, with the majority succumbing within months after diagnosis. Importantly, death of individuals with t-MNs is not only attributable to resistant primary or secondary diseases but is also affected by the toxicity of high-dose chemotherapy and HSCT, as evidenced by TRM rates of up to 60%. This is likely due to 'subclinical' organ damage following chemo- and/or radiotherapy for the antecedent disease. Additionally, although yet unproven, the hypersensitivity of normal cells of t-MN patients to cytotoxic treatments may also contribute to poor outcome as is well known for individuals with Fanconi anaemia, a constitutional disorder with impaired DNA damage response mechanisms and high propensity for developing AML (D'Andrea and Grompe, 2003). Implementing risk scores as proposed by the EBMT and CIBMTR will help to select patients with t-MNs eligible for intensive treatment strategies. Similar to *de novo* myeloid malignancies, the evaluation of novel treatment approaches that target constitutionally activated pathways or epigenetically modified loci in leukaemic cells may improve treatment efficacy in t-MN patients as well.

As the number of patients with t-MNs is expected to rise, safety issues of cytotoxic therapies are becoming increasingly important (Pugsley *et al.*, 2008). Several strategies have been employed to reduce the risk for therapy-related malignancies without compromising success rates for the respective primary disorders. For example, in patients with HD, replacement of the leukaemogenic MOPP regimen (mechlorethamine, vincristine, procarbazine and prednisone) by COPP (mechlorethamine substituted by cyclophosphamide) and ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) significantly reduced therapy-related MDS and AML cases (Andrieu *et al.*, 1990; Delwail *et al.*, 2002; Schonfeld *et al.*, 2006). Another promising approach to minimize cumulative doses of conventional chemo- and radiotherapy and to ameliorate acute and late toxicities is introducing antibodies, small molecule inhibitors and other targeted therapies into antineoplastic regimens. An alternative way to enhance the safety of cytotoxic therapies is adjusting treatment regimens based on a patient's individual genetic profile. While the relevance of genetic variants associated with toxicities of chemo- and/or radiotherapy awaits evaluation in prospective cohort studies, this personalized approach could provide the tools needed for proper t-MN risk assessment. Combined with novel targeted treatments, SNP profiles could help reduce the incidence of severe consequences of antineoplastic

and immunosuppressive treatments without threatening therapeutic success rates for the primary disorders.

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Conflict of interest

None.

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