Stem Cell Reports

Review

Clonal Hematopoiesis of Indeterminate Potential as a Novel Risk Factor for Donor-Derived Leukemia

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https://doi.org/10.1016/j.stemcr.2020.07.008

Hematopoietic stem cell transplantation (HSCT) is a critical treatment modality for many hematological and non-hematological diseases that is being extended to treat older individuals. However, recent studies show that clonal hematopoiesis of indeterminate potential (CHIP), a common, asymptomatic condition characterized by the expansion of age-acquired somatic mutations in blood cell lineages, may be a risk factor for the development of donor-derived leukemia (DDL), unexplained cytopenias, and chronic graft-versus-host disease. CHIP may contribute to the pathogenesis of these significant transplant complications via various cell-autonomous and non-cell-autonomous mechanisms, and the clinical presentation of DDL may be broader than anticipated. A more comprehensive understanding of the contributions of CHIP to DDL may have important implications for the screening of donors and will improve the safety of HSCT. The objective of this review is to discuss studies linking DDL and CHIP and to explore potential mechanisms by which CHIP may contribute to DDL.

Introduction

Hematopoietic stem cell transplantation (HSCT) is an important treatment option for a variety of malignant and non-malignant hematological and non-hematological diseases. In autologous transplantation, bone marrow (BM) from a patient is harvested before myeloablation and is then re-infused into the same patient. In contrast, allogeneic transplantation replaces the recipient's BM with donor BM after myeloablation. A key feature of HSCT is its reliance on the ability of hematopoietic stem and progenitor cells (HSPCs) to reconstitute the BM and to undergo normal hematopoiesis. Despite the utility of HSCT, a variety of complications can occur, including infection, secondary malignancy, and relapse (Hatzimichael and Tuthill, 2010). Originally pioneered in children, HSCT is now being used in older patient populations (Muffly et al., 2017). However, the incidence of relapse in elderly patients undergoing allogeneic HSCT is 35–40% after 1–2 years, inferring that relapse may be a significant concern (Hsu et al., 2020). While the extension of HSCT to older patient populations offers a valuable new treatment option, it is important to understand the risk factors that may influence transplant outcomes in this population.

Donor-derived leukemia (DDL) is a severe complication of HSCT in which patients later develop a secondary leukemia of donor origin (Fialkow et al., 1971; Wiseman, 2011). Previously considered rare, DDL mayconstitute 5% or more of leukemia relapses, and the number of cases has continued to increase over the last 50 years, particularly since 2000 (Suarez-Gonzalez et al., 2018a; Wiseman, 2011). The median age of recipients with DDL ranges from 31 to 53 years, indicating that it may be more common in adults (Suarez-Gonzalez et al., 2018a; Wang et al., 2011; Wiseman, 2011). DDL is distinguished from traditional relapse as it frequently exhibits different cytogenetic and phenotypic characteristics from the original disease, leading to considerable disease heterogeneity. Acute myeloid leukemia (AML) accounts for about 50% of cases, while acute lymphoid leukemia (ALL) and myelodysplastic syndrome (MDS) represent 23% and 20% of cases, respectively (Suarez-Gonzalez et al., 2018a; Wang et al., 2011; Wiseman, 2011). Notably, while traditional relapses present shortly after HSCT, DDL often exhibits a longer latency with a median time to diagnosis of 26 months, implying that significant changes must occur to promote DDL (Suarez-Gonzalez et al., 2018a; Wiseman, 2011). The heterogeneity of DDL and its origin from donor cells provide diagnostic and treatment challenges, and DDL patients often exhibit poor prognoses (Wiseman, 2011). Mortality has been estimated at 47% within a median follow-up period of 8.5 months, and the median time from diagnosis to death is 10.6 months, underscoring the importance of elucidating the causative factors of DDL and of developing effective therapies (Suarez-Gonzalez et al., 2018a; Wang et al., 2011). While its underlying etiology is not known, contributing factors may include preleukemic donor cells, cytotoxic chemotherapy, bystander radiation damage, defective BM microenvironment, impaired immune surveillance, replicative stress, viral and/or oncogene integration, and occult leukemia in the donor (Wiseman, 2011). Collectively, these studies demonstrate that DDL is a significant adverse event of an important treatment option and that a better understanding of its pathogenesis and risk factors will improve both diagnosis and treatment.

Clonal Hematopoiesis of Indeterminate Potential Represents a Novel Risk Factor for DDL

Clonal hematopoiesis of indeterminate potential (CHIP) is characterized by the expansion of specific leukocyte clones in healthy individuals (Jaiswal et al., 2014). As individuals age, they accumulate somatic mutations in their HSPCs that give rise to different blood cell lineages, including monocytes, neutrophils, B cells, T cells, and megakaryocytes. These mutations can endow a subpopulation of these cells with a growth advantage (Jaiswal and Ebert, 2019). Using a variant allele frequency (VAF) of 2% or greater in otherwise healthy individuals, the incidence of CHIP may be as high as 95% depending on the method of detection used, demonstrating that it may be quite common (Steensma, 2018b; Young et al., 2016). The three most commonly mutated genes in CHIP are the epigenetic regulators *Tet2 methycytosine dioxygenase 2* (*TET2*), *DNA methyltransferase 3A* (*DNMT3A*), and *Associated sex combs-like 1* (*ASXL1*) (Jaiswal et al., 2014). Importantly, CHIP is associated with increased risks of hematological malignancy, cardiovascular disease (CVD), and all-cause mortality (Jaiswal et al. 2014, 2017). The presence of these preleukemic mutations in donor BM cells may increase the potential for these cells to undergo oncogenic transformation, suggesting that CHIP may be an important risk factor for DDL if transmitted to recipients.

CHIP may contribute to altered transplant outcomes and the development of DDL after both autologous and allogeneic HSCT (Table 1). Autologous transplantation can facilitate expansion of HSPC clones containing CHIP-associated mutations (Ortmann et al., 2019). These HSPCs demonstrate long-term engraftment and reconstitute the blood cell lineages. CHIP patients exhibited longer neutrophil reconstitution, longer periods of hospitalization, and prolonged neutropenia. Clones carrying these preleukemic mutations can be detected years before disease onset and expand in response to transplantation (Berger et al., 2018; Ortmann et al., 2019). The stress of transplantation has been proposed to drive expansion of HSPCs containing CHIP-associated mutations, implicating factors in the transplant environment in the modulation of HSPCs containing CHIP-associated mutations (Ortmann et al., 2019). In patients who underwent autologous transplantation for lymphoma, CHIP correlated with inferior survival and an increased risk of therapy-related myeloid neoplasms (TMNs) (Gibson et al., 2017b). Notably, patients carrying more than one mutation at transplantation exhibited an increased risk of TMN. In a similar study, decreased overall survival was specifically observed in patients carrying mutations in DNA repair pathway genes, such as *protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D* (*PPM1D*), *tumor protein 53* (*TP53*), *BRCA1/BRCA2-containing complex subunit 3* (*BRCC3*), and *RAD21 cohesin complex component* (*RAD21*)

(Husby et al., 2020). *PPM1D* can drive clonal hematopoiesis (CH) in response to cytotoxic therapy, inferring a potential role for external stressors in promoting clonal expansion (Hsu et al., 2018). However, chemotherapy may not be required, providing the opportunity for multiple contributing factors (Ortmann et al., 2019). Surprisingly, mutations in other CHIP-associated genes, such as *DNMT3A*, *TET2*, and *ASXL1*, were observed but were not associated with inferior outcomes, implying that specific CHIP-associated mutations may be more susceptible to the autologous transplant environment (Husby et al., 2020). Similarly, in patients undergoing autologous transplantation for multiple myeloma, CHIP correlated with decreased survival and increased multiple myeloma progression, indicating that CHIP can modulate multiple types of hematological malignancy (Mouhieddine et al., 2020). Autologous transplantation in CHIP patients is also associated with late non-relapse mortality, demonstrating that CHIP-associated mutations have diverse effects on transplant outcomes (Slavin et al., 2019).

In allogeneic transplantation, CHIP also correlates with altered transplant outcomes. Unexplained cytopenias were observed in HSCT recipients of CHIP donors, demonstrating that donor clones containing CHIP-associated mutations can be transmitted to transplant recipients and can influence recipient blood cell populations (Gibson et al., 2017a). CH is commonly detected in both donors and recipients, and these transmitted clones can expand in recipients (Boettcher et al., 2020; Wong et al., 2020). The presence of unexplained cytopenias is more common in recipients with older donors, consistent with a possible role for CHIP in pathogenesis (Gibson et al., 2017a). While cytopenias are observed in DDL cases, it is not known if they contribute to DDL pathogenesis (Wang et al., 2011). HSPCs from older CHIP donors can also increase the risk of chronic graft-versus-host disease (cGVHD), a multi-system inflammatory response that occurs about 100 days after transplant (Frick et al., 2019; MacDonald et al., 2017). The longer latency of cGVHD is consistent with the later presentation of DDL, compared with traditional relapse. Surprisingly, CHIP-associated mutations of donor origin also correlate with a reduced occurrence of relapse (Frick et al., 2019). It was postulated that, due to their enhanced growth advantage, HSPCs bearing CHIP-associated mutations outcompete the residual leukemic HSPCs that can cause traditional relapse. Indeed, cGVHD may be inversely related to the occurrence of primary disease relapse; however, it may also create an immunosuppressive environment in which leukemia and other cancers can arise (Poonsombudlert et al., 2019; Rizzo et al., 2009).

Similarly, MDS, a clonal disorder characterized by BM failure that frequently progresses to AML, may be an early or intermediary phase in progression to DDL, suggesting

Table 1. Studies Reporting a Role for CHIP in the Outcomes of Both Autologous and Allogeneic HSCT

Study	No. of Patients	Donor/Recipient Ages	Transplant Type	DDL Cases	Clinical Outcomes
Gondek et al. (2016)	61 donor/recipient pairs	>60/43 and 26 years (in two DDL cases)	allogeneic; DDL cases involved related donors	2	Focal erythroid dysplasia, myeloid blasts, cytopenias, and MDS were observed in DDL patients.
Gibson et al. (2017a, 20170b)	552 donor/recipient pairs	40–66/51–68 years in cases with cytopenias	allogeneic; related and unrelated donors	No cases reported	89 patients exhibited cytopenias 6 of which were unexplained.
Herold et al. (2017)	1 donor/recipient pair	54/43 years	allogeneic; related donors	1	The recipient developed DDL, while the donor later developed AML.
Frick et al. (2019)	500 donor/recipient pairs	65/60 yrs (median age)	allogeneic; related donors	2	Increased chronic GVHD and decreased relapse correlated with CHIP. *DNMT3A* mutations associated with a decreased risk of relapse.
Gibson et al. (2017b)	401 patients	170 patients ≥60 years; 231 patients <60 years	autologous	18 cases of TMN	Patients with CHIP exhibited an increased risk of TMN, decreased overall survival, and increased mortality due to CVD.
Ortmann et al. (2019)	81 patients with solid tumors or lymphoid disease	mean age of 55.4 years at graft collection	autologous	No cases reported; CHIP clones exhibited expansion.	Patients with CHIP demonstrated longer neutrophil reconstitution after transplant, longer periods of hospitalization, and prolonged neutropenia.
Boettcher et al. (2020)	45 donor/recipient pairs	37/38 years initially; 57/61 years at study inclusion (median age)	allogeneic; related donors	1 MDS case	Donor-engrafted clonal hematopoiesis was detected in 5 cases . One case progressed to MDS.
Wong et al. (2020)	25 donor/recipient pairs	20–58/19–69 years	allogeneic; unrelated donors	No cases reported.	Clonal expansion, but not DDL, was observed during the follow-up period.
Husby et al. (2020)	892 lymphoma patients	≥18 years	autologous	CHIP correlated with an increased risk of TMN.	Patients with mutations in DNA damage repair genes exhibited worse overall survival and increased intensive care admissions.
Mouhieddine et al. (2020)	629 multiple myeloma patients	58 years	autologous	21 CHIP patients developed MDS or AML after transplant.	CHIP patients had worse overall survival and progression-free survival, primarily due to progression of multiple myeloma. CHIP was not associated with an increased risk of TMN.

CHIP, clonal hematopoiesis of indeterminate potential; TMN, therapy-related myeloid neoplasm; MDS, myelodysplastic syndrome; HSCT, hematopoietic stem cell transplant; CVD, cardiovascular disease.

that DDL may present along a spectrum of disease progression (Sperling et al., 2017; Suarez-Gonzalez et al., 2018a; Wiseman, 2011). MDS has been observed in up to 70% of DDL cases, and CHIP-associated MDS after allogeneic transplantation has been reported (Boettcher et al., 2020; Schwartz et al., 2018; Wang et al., 2011). Loss of *Asxl1* in

mice causes an MDS-like phenotype, and somatic mutations are associated with inferior outcomes in MDS patients undergoing HSCT, implicating CHIP-associated mutations in MDS (Abdel-Wahab et al., 2013; Bejar et al., 2014). Germline mutations, including those in *GATA binding protein 2* (*GATA2*), *DEAD-box helicase 41* (*DDX41*), *CCAAT enhancer binding protein alpha* (*CEBPA*), and *runt-related transcription factor 1* (*RUNX1*), can also cause donor-derived AML and MDS; however, the implications of CHIP in patients with these germline mutations has not yet been explored (Berger et al., 2017; Galera et al., 2018; Owen et al., 2008; Xiao et al., 2011). In particular, DDX41 mutations have been observed in older DDL patients together with mutations in CHIP-associated genes, providing an opportunity for interactions between germline mutations and CHIP-associated mutations in DDL (Berger et al., 2017).

As clonal evolution and the acquisition of mutations contribute to leukemogenesis, additional events may be required to drive CHIP-mutant HSPCs toward DDL (Leeksma et al., 2019; Sandén et al., 2020; Schaefer and Lindsley, 2018). Patients with CHIP-associated DDL exhibit multiple mutations in blood cell lineages and complex clonal compositions (Boettcher et al., 2020; Gondek et al., 2016; Herold et al., 2017; Suarez-Gonzalez et al., 2018b). The combinations of clones that arise and the specific mutations that they carry may influence their leukemic potential (Wong et al., 2018). The presence of additional mutations in clones originally containing CHIP-associated mutations and the evolution of independent clones with new mutations have been detected, highlighting the complexity of clonal evolution (Boettcher et al., 2020). The combination of loss of *Tet2* with constitutive activation of the *fms related tyrosine kinase* (*Flt3*) alters the chronic myelomonoctyic leukemia- or myeloproliferative neoplasm-like phenotype characteristic of single mutations to an AML-like phenotype (Ramdas et al., 2020; Shih et al., 2015). This finding demonstrates that additional mutations can modify the disease presentation and may help explain the heterogeneity observed in DDL. The presence of both germline and somatic mutations may further enhance clonal complexity in the context of DDL. The clonal evolution of mutant HSPCs is also consistent with the longer latency observed in DDL compared with traditional relapse, indicating that time is needed to acquire these mutations. Consistent with the observation that clones carrying CHIP-associated mutations may persist for years before progressing to DDL, somatic mutations can be present years before the diagnosis of AML (Desai et al., 2018). Notably, in many of these studies, donor mutations in *DNMT3A* are frequently transmitted to recipients and contribute to cGVHD, clonal evolution, and DDL, supporting a unique role for *DNMT3A* (Boettcher et al., 2020; Frick et al., 2019; Gibson et al., 2017a; Gondek et al.,

2016; Herold et al., 2017; Yasuda et al., 2014). CHIP-associated mutations in different genes may have differential propensities to affect HSCT and promote DDL; however, additional studies are needed. Collectively, these studies suggest that CHIP-associated mutations of donor origin can alter HSCT outcomes and may provide the context in which DDL can develop. The ability of HSPCs carrying CHIP-associated mutations to impair graft function indicates a need for further studies to evaluate the incidence of CHIP in transplant failure. cGVHD and MDS may be intermediary stages in the development of DDL that can progress through clonal evolution and may differ from their traditional presentations in the context of CHIP (Figure 1). However, it is not known if CHIP-associated cGVHD or MDS can progress to DDL and, if so, which factors promote this transition.

Both Cell-autonomous and Non-cell-autonomous Factors May Contribute to DDL

The "seed and soil" hypothesis is an important concept in DDL and may be relevant in considering the role of CHIP in DDL (Flynn and Kaufman, 2007). It highlights the potential contributions of both intrinsic and extrinsic factors to leukemogenesis. One hypothesis regarding DDL pathogenesis is that HSPCs acquire preleukemic mutations due to the stresses of the transplantation environment (Flynn and Kaufman, 2007; Wiseman, 2011). However, in CHIP, HSPCs already carry preleukemic mutations and may be primed to promote DDL. The ability of clones containing CHIP-associated mutations to expand within the HSPC population supports an inherent growth advantage for these mutations. Cell-autonomous properties of HSPCs bearing CHIP-associated mutations may depend on the epigenetic state of these cells and may be retained under stressful conditions, including transplantation, inflammation, and genotoxic stress, implicating intrinsic features of HSPCs in clonal behavior during DDL (Yu et al., 2016). The presence of germline mutations in donor HSPCs may also endow these cells with cell-autonomous advantages.

CHIP-associated mutations in different epigenetic regulators can bias the differentiation potential of HSPCs, leading to different clinical presentations (Xie et al., 2014). Similarly, inactivation of *Tet2* and *Asxl1* in mice results in myeloid pathologies, whereas loss of *Dnmt3a* causes both lymphoid and myeloid disease patterns (Abdel-Wahab et al., 2013; Li et al., 2011; Mayle et al., 2015). Consequently, these mutations may influence the presentation of DDL. In a cohort of CHIP patients, higher VAFs were detected in granulocytes, monocytes, and natural killer cells, compared with B and T cells, indicating a propensity for CHIP-associated mutations in myeloid lineages (Arends et al., 2018). However, in DDL patients, CHIP-associated mutations are detected in both myeloid and lymphoid
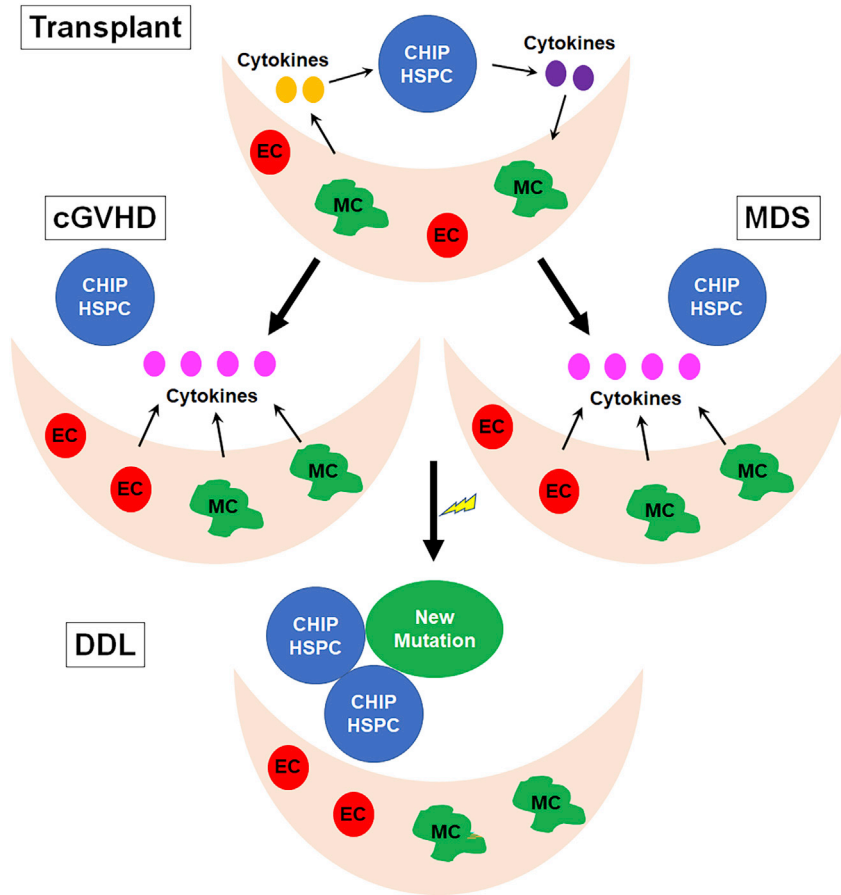
Figure 1. cGVHD and MDS May Represent Intermediary Stages in the Development of DDL

Both HSPCs containing CHIP-associated mutations and the aging, conditioned, and leukemic BM microenvironment can cause inflammation through the secretion of cytokines and may lead to cGVHD or MDS. These inflammatory states may facilitate expansion of CHIP clones, acquisition of new mutations, or changes in hematopoiesis that promote DDL. Additional triggers (lightning bolt), such as comorbidities, may also be needed to progress to DDL. EC, endothelial cells; MC, mesenchymal cells.

lineages, supporting the presence of these mutations in HSPCs (Boettcher et al., 2020; Herold et al., 2017). Intriguingly, in a 7-year longitudinal study of a single patient, only the myeloid lineage acquired additional mutations and progressed to leukemia (Herold et al., 2017). Consistently, most DDL cases cause AML; however, as DDL also includes lymphoid leukemias, a distinct mechanism may exist for the development of donor-derived lymphoid leukemias (Suarez-Gonzalez et al., 2018a; Wiseman, 2011). It is not yet known if CHIP contributes to both myeloid and lymphoid presentations of DDL. Germline mutations, such as those in *RUNX1*, are associated with myeloid malignancies, and may, therefore, further bias HSPCs toward myeloid lineages in DDL (Owen et al., 2008). As CHIP-associated mutations are typically observed in older individuals, intrinsic factors of aged HSPCs may also contribute to skewing of differentiation toward myeloid lineages (Mendelson and Frenette, 2014). In addition to influencing expansion and differentiation, CHIP-associated mutations can influence signaling in HSPCs and their progeny. For example, loss of *Tet2* and *Dnmt3a* in mice promotes inflammation (Cai et al., 2018; Cull et al., 2017; Leoni et al., 2017; Zhang et al., 2015). The release of cytokines by *Tet2*-defi-

cient HSPCs alters the frequencies of mesenchymal stem cells, endothelial cells, and osteoblasts and may make the BM microenvironment more conducive to DDL (Li et al., 2018; Ramdas et al., 2020) (Figures 1 and 2). Together, these studies demonstrate that HSPCs bearing CHIP-associated mutations may intrinsically contribute to DDL pathogenesis via multiple mechanisms.

While preleukemic mutations can provide donor HSPCs with cell-autonomous advantages and signaling mechanisms, the BM microenvironment promotes leukemogenesis via non-cell-autonomous mechanisms that may also be relevant in DDL (Schepers et al., 2015). Consisting of diverse cell populations, including osteoblasts, mesenchymal cells, and endothelial cells, the BM microenvironment provides signaling and secreted factors that facilitate the engraftment of donor HSPCs (Mendelson and Frenette, 2014). However, conditioning regimens can radically alter the BM microenvironment, damaging the stromal cells and impairing their ability to support hematopoiesis during recovery (Banfi et al., 2001; Domenech et al., 1998). Significant changes in the gene expression and DNA methylation of multipotent mesenchymal stromal cells and HSPCs are

detected in recipient BM up to 1 year after transplant (Shipounova et al., 2017; Trino et al., 2019). The potential role for methylation in the transplant BM microenvironment is particularly compelling considering that *DNMT3A* mutations are frequently present in CHIP-associated DDL.

Inflammation is also an important regulator of the BM microenvironment during transplantation (Singh et al., 2020). Interleukin-1β (IL-1β) is secreted by stromal cells in response to irradiation and can modulate both mesenchymal cell proliferation and hematopoiesis (Bigildeev et al. 2013, 2015). Importantly, its ability to regulate hematopoiesis may be context-dependent (de Haan et al., 1993). Transplantation stresses may create an environment that selects for HSPCs bearing CHIP-associated mutations from the transplanted BM. Consistent with this notion, inflammation facilitates the expansion of *Tet2*-deficient HSPCs (Abegunde et al., 2018; Cai et al., 2018). In addition to conditioning, the BM microenvironment in transplant patients has been exposed to hematological malignancy and, therefore, may already be altered. For example, AML can remodel the BM microenvironment to support leukemic cells and suppress normal hematopoiesis (Baryawno et al., 2019; Kumar et al., 2018). The extent of this remodeling may affect the likelihood of transplanted HSPCs bearing CHIP-associated mutations to progress to DDL. Compared with primary hematological malignancies associated with CHIP the transplantation environment and previous exposure to hematological malignancy are unique stresses to HSPCs carrying CHIP-associated mutations in DDL and may shape the potential of the BM microenvironment and donor HSPCs to promote DDL.

Age alters the BM microenvironment and may affect the efficiency of HSCT in elderly patients (Ho et al., 2019; Verovskaya et al., 2019). Together with metabolic and epigenetic shifts, the composition of supporting cells and the levels of secreted factors in the BM microenvironment change with age (Mendelson and Frenette, 2014; Verovskaya et al., 2019). Detected both systemically and in the BM stroma, inflammation is an important event during aging and can alter hematopoiesis (Kovtonyuk et al., 2016; Helbling et al., 2019). As inflammation can contribute to cancer pathogenesis, one possible explanation for the development of DDL from CHIP donors is that hematopoietic lineages exhibiting pro-inflammatory phenotypes may modify the BM microenvironment and hematopoiesis to promote leukemogenesis (Figure 2) (Henry et al., 2015; Leimkuhler and Schneider, 2019). For example, chronic exposure to IL-1 reduces HSPC self-renewal capacity, skews differentiation toward myeloid lineages, hinders transplantation, and contributes to hematological malignancy (Pietras et al., 2016; Arranz et al., 2017). As cytokines regulate hematopoiesis, the perturbation of cytokine signaling associated with CHIP-associated HSPCs, transplantation, and aging may alter the hematopoietic potential of the transplanted donor cells (Figure 2) (Mirantes et al., 2014). Leukemic HSPCs exhibit higher self-renewal capacity when transplanted in adult mice, compared with neonates, suggesting that the aged BM microenvironment more effectively promotes leukemogenesis (Lee et al., 2019). It is unclear if the clinical presentation and pathogenesis of DDL differ with age and the mechanisms by which HSPCs bearing CHIP-associated mutations interact with the BM microenvironments of different ages are not known (Wang et al., 2011; Wiseman, 2011). Recently, CHIP-associated mutations were detected in much younger individuals (Wong et al., 2020). With these younger individuals as donors, efficient engraftment and expansion of clones bearing CHIP-associated mutations were reported, but DDL or GVHD were not observed up to 100 days after transplant. A longer latency, additional environmental factors, or aged HSPCs may be needed to drive progression to DDL or GVHD. The impact of and the mechanisms by which the combined stresses of aging and transplantation affect the BM microenvironment are not yet known. Transplantation and HSPCs carrying CHIP-associated mutations may further exacerbate and prolong age-associated inflammation, and this process may support DDL pathogenesis (Kovtonyuk et al., 2016).

Inflammation in the BM microenvironment can also cause DNA damage, which may drive the acquisition of additional mutations that can promote DDL (Wiseman, 2011; Leimkuhler and Schneider, 2019). Inflammation in BM stromal cells exacerbates genotoxic stress and promotes progression of preleukemic conditions (Zambetti et al., 2016). HSPCs carrying CHIP-associated mutations may be more susceptible to the acquisition of new mutations, facilitating the clonal evolution needed to progress to DDL (Pan et al., 2017). Seventy-two percent of DDL cases exhibit abnormal karyotypes, and CHIP donors and their recipients have shorter telomeres, supporting a role for genotoxic stress in CHIP-associated DDL (Boettcher et al., 2020; Suarez-Gonzalez et al., 2018a). As inflammation contributes to both cGVHD and MDS, these inflammatory states may provide the necessary stresses to drive the acquisition of additional mutations and transformation to DDL (Figure 1) (MacDonald et al., 2017; Sallman and List, 2019). The aging BM microenvironment also exhibits increased levels of reactive oxygen species, which can cause genotoxic stress in HSPCs (Verovskaya et al., 2019). Furthermore, in cases of CHIP-associated TMN, mutations in *TP53* and *PPM1D*, key regulators in DNA damage repair, are prominent and can cause clonal expansion (Chen et al., 2019; Gibson et al., 2017b). As germline mutations are associated with MDS and AML, the presence of these
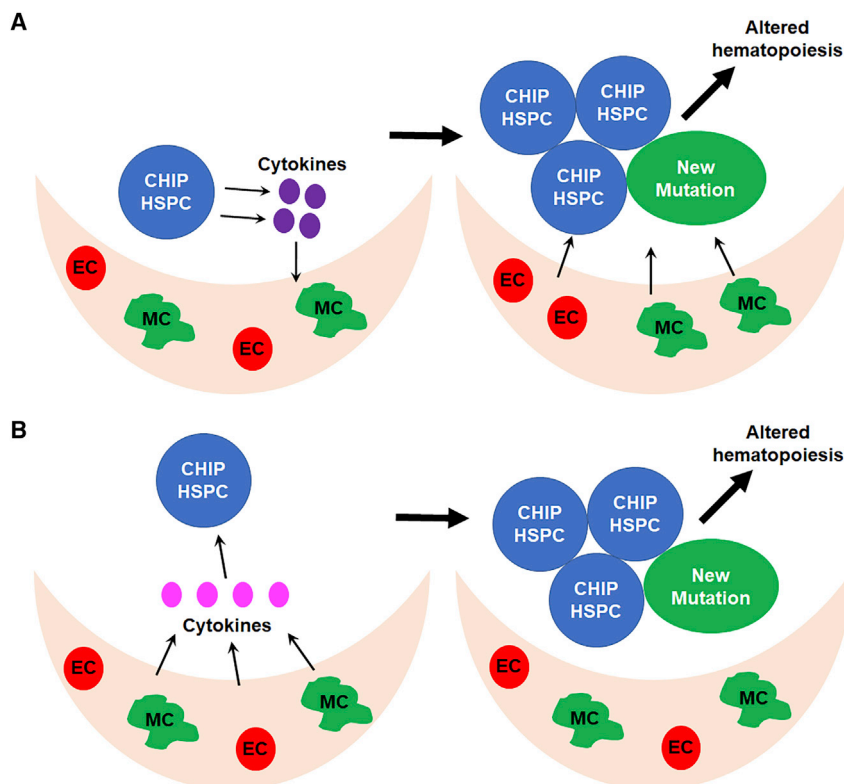
Figure 2. Models for the CellAutonomous and Non-cell-autonomous Factors that May Promote CHIP-Associated DDL

(A) In a cell-autonomous context, HSPCs carrying CHIP-associated mutations may release cytokines that can remodel the BM microenvironment to promote the development of DDL by altering normal hematopoiesis, by facilitating the expansion of CHIP clones, and/or by driving the acquisition of new mutations.

(B) In a non-cell-autonomous scenario, the aged, conditioned, or leukemic BM microenvironment of the recipient can secrete cytokines to promote the expansion of donor HSPCs containing CHIP-associated mutations, the acquisition of new mutations, and/or the disruption of normal hematopoiesis, which may lead to DDL. EC, endothelial cells; MC, mesenchymal cells.

mutations in the recipient BM microenvironment may also create an environment conducive to DDL (Kobayashi et al., 2017).

Likely, a combination of cell-autonomous and non-cell-autonomous factors is involved in DDL pathogenesis, and additional studies are needed to elucidate the importance of these factors in disease progression (Figure 2). Inflammatory signals may originate from HSPCs bearing CHIP-associated mutations, from the aged transplant BM microenvironment, or both, and significant communication may occur between the CHIP-mutant HSPCs and the BM microenvironment. These inflammatory signals may lead to cGVHD, MDS, and/or DDL. Our group recently showed that preleukemic *Tet2*-deficient HSPCs can modulate the BM microenvironment via pro-inflammatory factors and that these cells cause myeloproliferative neoplasms when transplanted into a leukemic environment (Ramdas et al., 2020). These findings demonstrate that the HSPCs carrying CHIP-associated mutations and the BM microenvironment may act in concert to drive DDL.

In further support of the role of the BM microenvironment, not all predisposed individuals develop CHIP-associated malignancy (Boettcher et al., 2020; Gondek et al., 2016; Wiseman, 2011). For example, a recipient can develop DDL, while the donor does not exhibit hematological malignancy, inferring that the recipients' BM microenvironment may stimulate HSPCs with CHIP-associated mutations to promote DDL. Alternatively, the donor and the recipient can present with different hematological malignancies and clinical outcomes. In 73 donor-recipient pairs in which the recipients developed DDL, 85% of the donors remained healthy, while 12% and 3% exhibited hematological malignancies and non-hematologic cancers, respectively (Suarez-Gonzalez et al., 2018a). Consistent with the relatively low risk of developing CHIP-associated hematological malignancies, this study indicates that most donors do not develop cancer (Steensma, 2018a). However, two distinct leukemias with different clinical outcomes arose in a related donor-recipient pair followed over time (Herold et al., 2017). This case shows that despite the presence of common initial mutations, additional factors unique to each patient may modulate the evolution and presentation of leukemia.

As CHIP is modulated by comorbidities, other conditions may influence DDL and may account for differences in clinical presentation between donors and recipients (Dragoljevic et al., 2018; Zhang et al., 2019). In addition, different hematopoietic stressors may exert distinct effects on the expansion of clones carrying specific CHIP-associated mutations (Wong et al., 2018). The wide variety of potential stresses present in elderly patient populations may be responsible for the significant heterogeneity observed
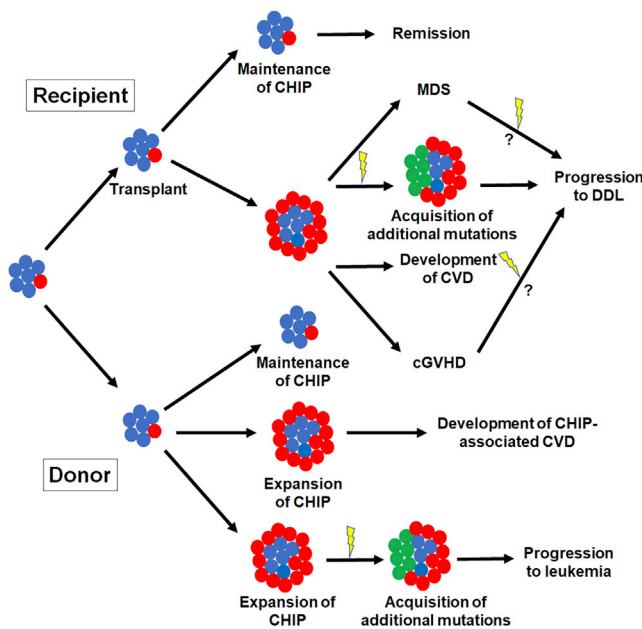
Figure 3. HSPCs Carrying CHIP-Associated Mutations Can Lead to Different Outcomes in Donors and Recipients

In both scenarios, mutant HSPCs can either maintain the size of the mutant clones (red) or these clones can expand. In donors, the expansion of CHIP clones can lead either to CVD or the acquisition of additional mutations (green). In recipients, the expansion of CHIP clones can lead to the acquisition of additional mutations, MDS, cGVHD, or the development of CVD. The acquisition of additional mutations likely requires a stressful event (lightning bolt) to drive mutagenesis. It is unclear whether CHIP-associated MDS and cGVHD can progress to DDL and whether additional mutations are needed.

in DDL patients (Figure 3). Inferior outcomes have been observed in elderly patients with comorbidities that undergo HSCT; however, it is not known if DDL and CHIP are more common in these patients (He et al., 2017; Muffly et al., 2017). These triggers imply that the causative factors of DDL may be more diverse than previously thought and that DDL should be considered in broader contexts clinically.

The Role of CHIP in DDL May Expand the Clinical Scope of DDL to Include Solid Organ Transplants, Non-hematologic Cancers, and CVD

As new causative and modulatory factors for DDL are being discovered, it may present in a variety of ways clinically. In addition to HSCT, DDL has been reported in cases of solid organ transplants (Bodo et al., 1999; Girsberger et al., 2013; Subklewe et al., 2004). A 57-year-old woman developed acute promyelocytic leukemia 2 years after receiving a liver transplant from a 16-year-old boy (Bodo et al., 1999). AML was observed in a 43-year-old man 3 years after a liver transplant

and in a 69-year-old kidney transplant recipient 2 years post-transplant (Girsberger et al., 2013; Subklewe et al., 2004). Notably, in the latter case, the donor was an 81-year-old woman with an unremarkable medical history. As in DDL due to HSCT, these cases exhibited long latencies to the development of leukemia, but it is not known if CHIP contributes to these manifestations of DDL. Significantly, the patients in these cases succumbed to their diseases, indicating that DDL from solid organ transplants is also severe (Bodo et al., 1999; Girsberger et al., 2013; Subklewe et al., 2004). The manifestation of DDL in these cases is particularly remarkable because the organs transplanted were not typically hematologic organs in adults, suggesting that other mechanisms may be involved in DDL. While the possible contribution of CHIP to DDL in solid organ transplants has not yet been investigated, the occurrence of DDL from solid organ transplants raises concerns about screening for CHIP in solid organ donors in addition to HSCT.

The identification of TMNs in CHIP patients undergoing autologous transplantation for lymphoma indicates that HSPCs containing CHIP-associated mutations may be particularly susceptible to cancer treatments and other environmental stresses (Gibson et al., 2017b; Husby et al., 2020). However, CHIP is also commonly detected in cancer patients before treatment and can predispose patients with non-hematologic cancers to the development of TMNs (Arends et al., 2018; Gillis et al., 2017; Takahashi et al., 2017). Patients with solid cancers undergoing treatment exhibited VAF changes of at least 50%, further supporting the sensitivity of hematopoietic cells bearing CHIP-associated mutations to external stresses (Arends et al., 2018). In addition, depending on the gene mutated, some patients needed more frequent blood cell transfusions and dose reductions, identifying a role for CHIP in guiding the treatment of non-hematologic cancers. These studies suggest that elderly cancer patients receiving treatment may be at an increased risk of developing DDL or other CHIP-associated conditions, especially if they have other comorbidities. Additional studies are needed to better understand these potential risk factors. These studies support a more expansive evaluation of CHIP in cancer patients generally.

While the risk of developing hematologic malignancies from CHIP is 0.5%–1%, there is a much greater risk of developing CVD (Dorsheimer et al., 2019; Jaiswal et al., 2017; Steensma, 2018a). Notably, CVD is also a significant morbidity in HSCT patients (Armenian et al., 2017). As in CHIP, HSCT patients exhibit heart failure and coronary artery disease; however, the mechanisms underlying this presentation are not known (Armenian et al., 2018). In a model of *Tet2* deficiency in atherosclerosis-prone *low-density lipoprotein receptor*-deficient mice, macrophages secreting IL-1β exacerbate atherosclerosis (Fuster et al., 2017). An increased risk of mortality due to CVD was

observed in CHIP patients undergoing autologous transplantation for lymphoma, inferring a possible role for CHIP in transplant-associated CVD (Armenian et al., 2017). Importantly, it is not known if CHIP increases the risk of CVD in DDL patients and if the presence of CHIP in an HSCT donor or recipient directly contributes to CVD. However, fewer CHIP-mutant cells may be required to promote CVD than hematological malignancy, highlighting the importance of evaluating CVD in HSCT patients and in patients with CHIP-associated DDL. Additional studies are needed to investigate these possibilities. Nonetheless, these studies infer a potential link between the hematopoietic and cardiovascular systems that may have significant implications for pathogenesis and the treatment of HSCT and DDL patients.

Collectively, these different clinical manifestations of DDL underscore a need to broaden the clinical approach to these patients both in diagnosis and treatment. A better understanding of the potentially diverse clinical presentations of these patients will facilitate their identification and proper diagnosis at earlier stages and promote the development of novel therapeutics.

CHIP Has Significant Implications for DDL

The potential of CHIP to contribute to DDL may also be a concern with other cell-based products, such as chimeric antigen receptor T cells and donor lymphocyte infusions (DLIs); however, more information is needed. With DLIs, the composition of both donor and recipient HSPC clones may evolve over time and respond to different stimuli, altering the factors influencing DDL pathogenesis. CHIP-associated inflammation may also have clinical implications. Our group has shown that inhibition of inflammation using the small-molecule APX3330 blocks clonal expansion of *Tet2*-deficient HSPCs (Cai et al., 2018). However, it is also important to understand the effects of anti-inflammatory agents on the BM microenvironment as both HSPCs and the BM microenvironment may be sources of inflammatory signals in CHIP-associated DDL.

Currently, screening of donors for CHIP is controversial (DeZern and Gondek, 2020; Gibson and Lindsley, 2020). Screening for donors with CHIP before transplantation may prevent adverse effects in HSCT patients as seemingly healthy individuals may harbor growth-promoting mutations in their HSPCs. However, challenges exist in the ability to effectively screen for all possible CHIP-associated mutations. As improved technologies facilitate the detection of CHIP at lower VAFs, the elucidation of a pathogenic threshold and its clinical significance are complex. The biological context of these mutations in the recipient and the influences of comorbidities may govern their behavior and clinical relevance. Screening for germline mutations is recommended to avoid related donors in these cases (Galera

et al., 2018; Kobayashi et al., 2017). While the consequences of germline mutations for CHIP-associated DDL are not yet known, future studies are needed to understand the implications of these mutations and cancer predisposition syndromes, such as neurofibromatosis, Bloom syndrome, Fanconi anemia, and Li-Fraumeni syndrome, for CHIP-associated DDL (Wiseman, 2011). In addition, the status of CH in the donor can evolve over time, supporting long-term follow-up of both donors and recipients. It also is not known if there are CHIP-independent mechanisms of DDL and if there are differences in clinical presentation, mutations, and prognosis in patients with CHIP-associated DDL, compared with DDL that is independent of CHIP. Ultimately, further studies are needed to understand the effectiveness of donor screening.

Conclusions

CHIP is a relatively common novel condition in elderly individuals and may be an important risk factor for the development of DDL, a significant complication of HSCT. The more frequent use of HSCT in older populations is particularly concerning as CHIP-associated mutations are frequently observed in individuals older than 65 years and increase with age. Recent studies suggest that CHIP may adversely affect transplant outcomes and promote DDL; however, many unanswered questions remain. CHIP may contribute to DDL pathogenesis via cell-autonomous and non-cell-autonomous mechanisms and may lead to novel presentations of donor-derived disease, such as CVD. A better understanding of the contributions of CHIP to DDL will provide important knowledge about the mechanisms of leukemogenesis and will have significant implications for the diagnosis, treatment, and the development of novel therapies for DDL.

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