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TET2 mutation as Prototypic Clonal Hematopoiesis Lesion

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Abstract

Loss of function *TET2* mutation (*TET2*^{MT}) is one of the most frequently observed lesions in clonal haematopoiesis (CH). TET2 a member TET-dioxygenase family of enzyme that along with TET1 and TET3, progressively oxidize 5-methyl cytosine (mC) resulting in regulated demethylation of promoter, enhancer and silencer elements of the genome. This process is critical for efficient transcription that determine cell lineage fate, proliferation and survival and the maintenance of the genomic fidelity with aging of the organism. Partial or complete loss-of-function *TET2* mutations create regional and contextual DNA hypermethylation leading to gene silencing or activation that result in skewed myeloid differentiation and clonal expansion. In addition to myeloid skewing, loss of *TET2* creates differentiation block and provides proliferative advantage to hematopoietic stem and progenitor cells (HSPCs). *TET2*^{MT} is a prototypical lesion in CH, since the mutant clones dominate during stress haematopoiesis and often associates with evolution of myeloid malignancies. *TET2*^{MT} clones has unique privilege to create and persist in pro-inflammatory milieu. Despite extensive knowledge regarding biochemical mechanisms underlying distorted myeloid differentiation, and enhanced self-replication of *TET2*^{MT} HSPC, the mechanistic link of various pathogenesis associated *TET2* loss in CHIP is less understood. Here we review the recent development in *TET2* biology and its probable mechanistic link in CH with aging and inflammation. We also explored the therapeutic strategies of targeting *TET2*^{MT} associated CHIP and the utility of targeting *TET2* in normal haematopoiesis and somatic cell reprograming. We explore the biochemical mechanisms and candidate therapies that emerged in last decade of research.

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Introduction

Circulating myeloid cells carrying somatic mutations characteristic of myeloid neoplasms (MN) occur in a significant proportion of otherwise healthy, mostly elderly individuals, a condition termed clonal haematopoiesis of indeterminate potential (CHIP). In recent years, CHIP has been of considerable interest of research due to its association with several pathophysiological conditions. Individuals with CHIP have increased risk (10-25X) of developing MN[1-5], including treatment-related leukemias in cancer survivors who received chemotherapy. Thus, CHIP is a condition characterized by the presence of a hematopoietic stem cell (HSC) clone harbouring a somatic mutation in individuals without evidence of hematologic malignancy (HM)[6]. Depending on type of mutations in certain genes age and other confounding conditions. CHIP occurs in 15% of people over 65 and ~30% in octogenarians, therefore often termed as age-related clonal haematopoiesis (ARCH). The prevalence of CHIP is strictly linked to age, with a low incidence before 50 years (1%), steadily increasing in elderly subjects (10% over 70 years)[1,4]. The etiology of CHIP is still object of study: no clear genetic predisposition has been shown for CHIP, whereas previous radio/chemotherapy, smoking habit and male gender have been identified as predisposing factors[7,8]. Several recent reports suggest that the age-related inflammatory microenvironment promotes the malignant evolution of CHIP, but detailed causative mechanisms have not been described. Although asymptomatic, CHIP is correlated with an increased risk of HM, a broad spectrum of cardiovascular and non-malignant conditions, and all-cause mortality[1-5].

Apart from *DNMT3A* and *TET2,* several studies from large cohorts demonstrated the presence of *ASXL1, TP53, JAK2, PPM1D, SF3B1, SRSF2, GNAS, BCORL1* and *CBL* mutations in significant proportion of CHIP cases[1,4]. The rate of progression to an HM among CHIP is estimated to be 0.5-1% per year[1,4]; however the progression rate differs among different type of mutations. In particular a mutation variant allele frequency (VAF) > 10% is significantly correlated with the development of HM (hazard ratio 49, as reported by Jaiswal et al.[1]). Furthermore, the most common CHIP drivers, *DNMT3A, TET2* and *ASXL1,* are usually detected at VAF of 10-20%, whereas other genes, such as *NRAS, KRAS, GNAS* are often detected at considerably lower VAF (0.1-0.5%)[8,9]. Finally, the mutations present different evolution over time with divergent clinical implications: Van Zeventer et al., analyzing a large cohort of older individuals, found that *DNMT3A* mutation don't show an increase of VAF over time, unlike *JAK2, TET2, ASXL1* and *TP53*. Furthermore, the maximum increase in VAF was detected in individuals harboring multiple mutations and the propensity to acquire additional mutations over time was higher in *TET2* and *ASXL1*-mutated patients compared to *DNMT3A*[10]. The most frequently observed genetic alterations (>50%) in CHIP/ARCH are loss of function (LOF) mutations in are in the genes *DNMT3A* and *TET2* that regulate epigenetic landscape of our genome. DNMT3A catalyzes methylation of DNA cytosine bases using cofactor S-adenosyl-L-methionine (SAM) as a methyl donor while TET2 catalyzes the iterative oxidation of methylated cytosine bases, using α-ketoglutarate, molecular oxygen O_2 and Iron (II) as cofactors followed by base excision repair (BER), of TET-dependent oxidation products 5fC and 5CaC, leading to cytosine demethylation. At first glance, these two genes most

commonly mutated in CHIP appear to have opposing functions: cytosine methylation and demethylation. Complexity and duality of the functional outcome in CHIP is poorly understood. Recent evidences suggest multiple gene mutation driven CHIPs rather than a single entity as a factor for malignant evolution. In this review we will primarily focus on *TET2*-driven CHIP, and its interaction with various pathogenic factors including aging, inflammation and stress hematopoiesis.

Structure and Function

Ten-eleven translocation (TET) family is a group of enzymes involved in epigenetic transcriptional regulation. TET1, TET2 and TET3, encoded by homonymous genes located on chromosomes 2p13.1, 41.24 and 10q21.3, respectively, present conserved cysteine-reach domain (CRD) and double-stranded β-helix (DSβO) fold domain at C-terminal; furthermore, TET1 and TET3, but not TET2, present a CXXC domain at N-terminal (Figure 1). TET2 CXXC domain, ancestrally present, was separated from the de gene due to chromosomal gene inversion during evolution. Thus, the binding to CpG dinucleotides in genes promotors, in TET1 and TET3 by CXXC domains, in TET2 is modulated by the separated gene IDAX (also known as CXXC4)[11].

TET proteins catalyse the conversion of 5mC (5-methylcytosine) to 5-hydroxymethylcytosine (5hmC) in a Fe(II)- and α-ketoglutarate (α-KG)-dependent manner (both linked to DsβO domain) and oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC): finally, 5caC bases are excised by thymine DNA glycosylase (TDG)[12,13]. Together, the oxidized methylcytosines catalysed by TETs are intermediates in DNA demethylation[14]. Furthermore, TET proteins can also directly interact with histones to regulate DNA transcription: in fact, they are able to bind O-linked β-N-acetylglucosamine (O-GlcNAc) transferase (OGT) to target gene promotors and regulate their transcription through histone H2B O-GlcNAcylation or proteolytic activation of host cell factor C1 (HCF1), part of Histone H3 lysine K4 (H3K4) methyltransferase complex SET1/COMPASS[15-18]. Furthermore, TET2, binding IκBζ (NF-kappa-B inhibitor zeta), can recruit Histone deacetylase 2 (HDAC2), leading to histone deacetylase and deregulation of IL-6 and PDL1[19,20] (Figure 1).

Intriguingly, few molecules were identified as regulators and interactors of TET proteins: Vitamin C (Ascorbic acid, AA) enhances TET-mediated 5mC oxidation, promoting thus demethylation[21,22]; microRNA miR-22 downregulates *TET2* gene expression with subsequent developing myelodysplastic syndromes (MDS) and HMs in mice models[23]; Uhrf1, a methyl-CpG-binding protein stimulates the activity of the TET1 in neuronal cells[24].

Similarity and differences among TET isoforms and their role in hematopoiesis

The most widely reported functions of TETs are their dioxygenase activity and its implications in maintaining an efficient transcription profile[25]. While TET1, TET2 and TET3 and their isoforms have great deal of similarity C-terminal catalytic domain they are vastly different in the structure and functions of N-terminal regulatory domain[25].

TET proteins are expressed in a broad spectrum of tissues[13] and all of them are expressed in hematopoietic tissue with varying expression levels depending on the lineage and the maturity stage[26]. However, as for the structure, also the function in physiological haematopoiesis differs among TET1/2/3, with overlapping functions.

TET1 is less expressed than TET2 and 3 and its expression is higher in HSCs and early and committed progenitors[26-28]. Studies about the predominant concentrations of TET2 or TET3 produced contrasting results[26,28,29]. However, the two isoform are differently expressed during haematopoiesis: TET2 is highly expressed both in HSC and in mature myeloid and lymphoid cells, whereas TET3 expression drops during differentiation[30].

As suggested also by their expression patterns, TET proteins have a pivotal role in the early haematopoiesis: in zebrafish models, Li et al. showed an overlapping role of Tet2 and Tet3 in regulating Notch1 in the physiological endothelium-to-HSC transition[28]. Studies on mice confirmed Tet central role in proper embryogenesis through the regulation of Lefty-Nodal pathway: inactivation of all the three Tet genes led to impairment of embryogenesis processes, such as abnormal maturation of axial mesoderm and failed specification of paraxial mesoderm, with a partial restoration introducing a single mutant allele of Nodal in the Tet mutant background[31]. Ma et al. proved the post-gastrulation Tet role showing that the loss of the three enzymes in endothelial cells (EC) at these stage brought to reduced number of HSC and progenitor cells and lethality in mid-gestation mouse embryos through hypermethylation and down-regulation of NFκB1 and other hematopoietic transcription factors (Gata1/2, Runx1, and Gfi1b)[32]. *TET1/TET2* double knock-out (*TET1/2*^{dko}) embryonic stem cells are viable and fertile with abnormal methylation and perturbed development[33]. Furthermore, Tet3^{ko} mice can develop to term but die at birth[34].

Although TET2 present a preponderant activity, also in late haematopoiesis overlapping and compensatory roles among TETs were observed. In particular TET2 carries out a regulatory and tumour suppressor activity by regulating DNA demethylation and chromatin modifications at enhancers and promotors of key genes which determine lineage commitment and differentiation[26].

Germline Tet2 deficient mice models, in fact, are viable and fertile but, as found by several authors, haematopoiesis goes through a skewed myeloid/lymphoid ratio, with expansion of granulocytes at expenses of lymphocytes; furthermore, the vast majority of *Tet2*^{ko} mice develop myeloid malignancies, with the rest developing lymphoid malignancies[15,29,35,36].

The study by Ito et al. on *Tet2-mutant* (*Tet2*^{Mut}) mice models confirmed the tendency of developing MDS-like syndromes but not lymphoid disorders[37]. Moreover, RNA sequencing analysis showed significant differences, between *Tet2*^{Mut} and *Tet2*^{ko} in expression of genes involved in cell proliferation, differentiation, gene regulation, and apoptosis. Among them, *Hoxa9* and *Gata2,* were significantly downregulated in *Tet2*^{ko}, but not in *Tet2*^{Mut}; as a confirmation, the overexpression of Gata2 in *Tet2*^{ko}, but not in *Tet2*^{Mut} bone marrow (BM) cells ameliorated disease phenotypes. Furthermore, *Tet1* was downregulated in both *Tet2*^{ko} and *Tet2*^{Mut} cells, whereas *Tet3* expression was slightly reduced only in *Tet2*^{ko} cells[37].

These observations brought to the conclusion of an additional key non-enzymatic role of TET2 in HSC homeostasis. Furthermore, the study by Pan et al. showed that both *Tet2*^{ko} and *Tet2* with abolished catalytic activity mice presented high rates of mutations occurrence; translated in clinical practice, the authors, analysing a large cohort of patients with myeloid malignancies, showed an higher rate of mutations in *TET2*^{Mut} patients versus wild type (*wt*)[38].

During myeloid lineage commitment, TET2 interacts with a broad variety of partners in order to regulate the accessibility to specific transcription factors, such as Cebpα, Irf8, Erg, and Runx1 for myeloid commitment and Gata1, Klf1 and Scl for erythroid differentiation[39,40]. TET2 regulates also myeloid-to monocyte/granulocyte, myeloid to-mast-cells, monocyte-to-dendritic cell and monocyte-to-osteoblast differentiation processes[41-43]. The key role in erythroid lineage development is also confirmed by Tet2^{ko} mice models, showing reduced red blood cells in peripheral blood and dysplastic erythropoiesis[35,44]. In human *TET2*^{ko} HSCs an increase of dysfunctional CFU-E progenitors was observed[45].

Furthermore, TET2 exert an additional anti-tumour activity repressing the production of monocyte/macrophage inflammatory cytokines, such as IL-1β, IL-6 and Arginase 1, involved in promoting proliferation and survival of mutant clones in myeloid malignancies and creating a leukemic niche in BM[46-48].

TET2 is also involved in lymphoid differentiation: it regulates proB-to-preB cell transition and final differentiation in plasma-cells, through upregulation of the plasma cell master regulators Prdm1 and Irf4[26,30]. Furthermore, above-mentioned evidence of lymphoid malignancies arising in *Tet2*^{ko} mice models, such as diffuse large B cell lymphomas, prove its tumour-suppressor activity in this cell lineage[49]. In a similar fashion, in T-cells, TET2 regulates CD4+ T helper cell and CD8+ memory T-cell differentiation[50]; in particular, through the upregulation of Runx2 and Runx3, it limits T follicular helper (Tfh) cells differentiation, as also confirmed by *TET2*^{ko} CD4^{+} naïve T cells, which preferentially differentiate into Tfh cells[30,51].

TET1 loss in *TET2*^{MT} HSPCs reprograms the differentiation circuit by *Tet2*^{ko} models after Tet1 deletion[52]. On the other hand, as TET2, also TET1 presents a lymphoid regulatory activity: Tet1/2^{dko} mice models develop B-cell malignancies[52] and T-cell specific double Tet1$^{/2dko}$ mice present autoimmune diseases[53].

TET3 presents key function also in adult haematopoiesis: mice models with Tet3-specific HSCs present a decrease number of HSCs in BM but normal composition of adult myeloid and lymphoid cells[30,54]. Furthermore, as shown by Gu et al., germline *Tet3*^{ko} mice models die at birth[34]. Cao et al., investigating the mechanism underpinning the impaired erythroid differentiation potential in *TET2* mutation, observed that, among *TET* genes, *TET3* was specifically upregulated in hypoxia conditions. In particular, the authors identified two HIF-1 (Hypoxia-Inducible Factor-1) binding sites in TET3 whose deletion abrogated the upregulation[55]. Accordingly, Yahn et al. observed an impairment of terminal erythroid differentiation in human *TET3*^{ko} HSCs[56].

Several authors also focused on TET2-TET3 overlapping activity particularly in murine model. In these models *Tet2/3*^{dko} mice present expansion of myeloid cells, accompanied by massive, progressive splenomegaly and hepatomegaly with impaired lymphoid and erythroid differentiation. Interestingly, TET3 loss of fucnction mutation single of in combination has not been observed in myeloid malignancies. Furthermore, 5hmC levels in *Tet2/3*^{dko} models were significantly decreased compared to the *wt* counterpart. Intriguingly, *Tet2*^{ko}/*Tet3*^{WT} and *Tet2*^{WT}/*Tet3*^{ko} models presented intermediate level of 5hmC and a milder myeloid disease phenotype when compared to *Tet2/3*^{dko}, with a longer median survival[30,57,58]. These observations suggest that the two isoforms can compensate for one another to suppress malignant transformation. On lymphoid compartment, the two isoforms cooperate in B-cell regulation at different steps of development: mice models with Tet2/3^{dko} early ProB cells develop B-ALL[59]. In pre-germinal centre (GC) B-cells, *Tet2/3* double loss brings to an impairment of plasma cell differentiation and lupus-like disease in mice whereas the ko in GC B-cells does not produce such phenotype[60]. In the same fashion, TET3 cooperates with TET2 in regulation of T-cell differentiation: mice models with T-cell harbouring *Tet2/3*^{dko} develop T-cell lymphoid malignancies whereas Tet2/3 combined loss in regulatory T-cells cause immune homeostasis impairment[61].

Taken together, these evidence suggest that TET proteins are fundamental epigenetic regulators in both early and late haematopoiesis, in a complex equilibrium of antagonist, compensatory, overlapping and redundant roles.

The many facets of TET2 CHIP

Apart from the extensively studied connection of *TET2*^{MT} CHIP with inflammation, aging and malignant progression, discussed in the following section, as a result of large genomic data sets analysis, *TET2*-driven CHIP was found to be linked to a broad range of conditions, with relatively poorly understood mechanistic underpinning (Figure 2).

The link to cardiovascular diseases was one of the firsts association emerged since genetic characterization of CHIPs: in 2017, Jaiswal et al highlighted the correlation of CHIP with higher risk of early-onset myocardial infarction, artery calcification and coronary heart disease[62]. Subsequently, Pascual-Figal et al. detected, in a small population of patients, an accelerated ischemic heart failure progression in terms of death and/or hospitalization in patient carrying *DNMT3A* or *TET2* mutation[63]. Bhattacharya et al., in a cohort of about 80.000 individuals, establish a connection between CHIP and risk of stroke irrespective of age, sex, and race. *TET2* showed the strongest association with total stroke and ischemic stroke, whereas *DMNT3A* and *TET2* were each associated with increased risk of haemorrhagic stroke[64]. To investigate the mechanistic nature or this phenomenon, Fuster et al. created a 10% *Tet2*^{ko} mice model which exhibited 60% larger plaques in the aortic root than wt controls: this observation was paralleled by an increase in total macrophage content in the atherosclerotic vascular wall. qRT-PCR analysis revealed that Tet2-deficient macrophages exhibit increased expression of pro inflammatory cytokines, chemokines, and enzymes. To mimic an in-vivo situation, the macrophages were then tested after exposure of oxidized low-density lipoprotein (oxLDL), tumour necrosis factor (TNF), and interferon γ (IFN-γ), stimuli present in atherosclerotic plaques. Strikingly,

only IL-1β resulted markedly up-regulated compared to wt controls. The blockade of NLRP3 inflammasome, multiprotein complex that cleaves pro—IL-1β to the active form, abrogated IL-1β secretion[65]. Furthermore, in the model by Sano et al., mice transplanted with 10% Tet2-deficient BM cells resulted more sensitive to angiotensin-II exposure in term of deterioration of cardiac function by echocardiography compared to *wt* controls. At the termination of the experiment, *Tet2*^{Mut} models presented also an increase in cardiac weight, cardiac myocyte cross sectional area and interstitial fibrosis[66].

TET2 affects cardiovascular homeostasis also through a complex regulation of EC functions and extracellular proteins. In fact, TET2 upregulates Robo4, a transmembrane protein which inhibits ECs via inhibiting Vascular endothelial growth factor (VEFG) and induce inflammation through IL-6 pathway and whose downregulation inhibits angiogenesis and is correlated to cardiovascular diseases[67-70]. TET2 also regulates the expression of Matrix metalloproteinase-9 (MMP9)[71], a protein with pleiotropic functions. MMP9 degrades extracellular matrix and promote restoring the heart tissues after insulting by mobilizing HSC from BM[72]; on the other hand, its overexpression is correlated to cardiac hypertrophy, vessel rarefaction, inflammation, and fibrosis[72,73]. Furthermore, TET2, through the interaction with forkhead box O3, enhances the expression of lipoprotein lipase (LPL), a key enzyme in lipid metabolism regulated by a variety of factors which may pose a pro-atherogenic or an anti-atherogenic effect[74].

Miller et al., in a large population of patients with chronic obstructive pulmonary disease (COPD), detected a correlation between CHIP and an increased risk of a more sever disease and decreased forced expiratory volume (FEV1). To investigate the mechanism underpinning these differences, the authors created *Tet2*^{ko} mice models which presented, after exposure to cigarette smoke, an accelerated development of emphysema compared to *wt* counterparts. Furthermore, *Tet2*^{ko} mice presented upregulation of IFN-I and IFN-II and lower transforming growth factor-β (TGF-β). The analysis of alveolar macrophages and monocytes also revealed a higher expression of inflammatory proteins[2].

Agrawal et al., analysing genomic data from nearly 200.000 individuals, detected a higher incidence of gout among CHIP patients, in particular *TET2*^{Mut} patients. Intriguingly, Tet2^{ko} mice models injected with intraperitoneal monosodium urate crystals presented higher IL-1β, C-C chemokine ligand 22 (CCL22), and C-C chemokine ligand 5 (CCL5) levels when compared to Tet2^{WT} controls. Furthermore, TET2 loss resulted in a more severe phenotype. Accordingly, macrophages from *Tet2*^{ko} mice presented a significantly higher IL-1β secretion[75].

A recent report described CHIP as risk factor for chronic liver disease; in particular, *TET2*-driven CHIP was associated with a fivefold increased risk of chronic liver disease. Furthermore, mice transplanted with *Tet2*^{ko} BM showed more lobular inflammation with prominent lymphoid aggregates and hepatocyte ballooning and increased liver fibrosis compared to *wt* controls, suggesting a role in *TET2* mutation in both development of steatohepatitis and progression to liver fibrosis. Interestingly, the authors individuated the liver macrophages, which expressed an inflammatory phenotype in *Tet2*^{ko} mice models, as the main mediator of liver injury[76].

Tobias et al., in an analysis of six prospective cohorts, found that, individuals with at study baseline were more likely to develop type 2 diabetes (T2D) irrespective of age, sex, body mass index (BMI), smoking, alcohol, education and self-reported race/ethnicity. Specifically, higher risk was for *TET2* and *ASXL1* mutations[77]. Furthermore, Oh et al. documented an association between *TET2* mutation and higher severity of Diabetic Peripheral Neuropathy in T2D[78]. The link between *TET2*-driven CHIP and glucose homeostasis impairment was investigated by Fuster et al. in mice models: Tet2 deficiency aggravated insulin resistance in both normally-aged mice and obese mice in a NLRP3 inflammasome-/IL-1β-mediated inflammation process fostered by activated macrophages[79].

In a large meta-analysis, Kestenbaum et al. found that CHIP was associated with greater risk of a 30% eGFR decline[80]; in a subsequent study by Vlasschaert et al., CHIP, and in particular not-DNMT3A-driven CHIP, emerged as risk factor associate with acute kidney injury. Mice models created to investigate this correlation revealed an increased mRNA expression of proinflammatory cytokines (Il1b, Tnf, ccl3, ccl2), increased kidney macrophage and neutrophil invasion and significantly increased interstitial fibrosis in *Tet2*^{ko} compared to *wt* controls[81].

TET2 mutation as maladaptive change in inflammation

As above-mentioned, a growing body of evidence points out a tight connection between aging, inflammation and survival advantage in *TET2*-mutated HSCs.

Several authors, through competitive BM cell transplantation and serial transplantation assays, observed an enhanced hematopoietic reconstitute capacity in *TET2*^{ko} HSCs[15,82]. Ostrander et al., comparing *TET2*^{ko} and *TET2 wt* HSC functions, confirmed an initial increase in self-renewal in the first ones, showing, though, a subsequent exhaustion at the same rate of *wt* ones. Intriguingly, the mutation sensitized HSCs to the acquisition of a common co-operating mutation (*Flt3*^{ITD}), associated with a more rapid expansion of committed progenitor cells[83].

More recent models highlighted the tight connection between *TET2* mutation and inflammatory context in malignancy development. Meisel et al., in fact, exploring the mechanism of penetrance of myeloid malignancies in *Tet2*^{ko} mice, found that asymptomatic *Tet2*^{ko} mice and *Tet2*^{ko} mice with myeloid malignancy presented similar HSC self-renewing potential, irrespective of health status, BM and splenic hematopoietic progenitors and superior to controls. Interestingly, the blockade of IL-6 significantly reduced the increased self-renewing capacity of *TET2*^{ko} progenitors in vitro without affecting normal controls; furthermore, in-vivo IL-6 blockade experiment demonstrated the requirements of IL-6 to develop a myeloid malignancy in *Tet2*^{ko} mice[84].

Yeaton et al. created an animal model Carrying a patient-derived *TET2* p.H1881R missense mutation; as previously described, *TET2* mutation cooperated with other somatic lesions (such as *FLT3*^{ITD}) to progress to myeloid malignancy. Interestingly, not all tumors carried additional somatic mutations in genes frequently mutated in hematologic malignancies, suggesting that progression to transformation can be in some cases independent of

acquisition of secondary mutations. Additionally, using single-cell analysis, the authors discovered that inflammatory signals alter the development of *TET2*^{Mut} cells with the differentiation skewing toward a monocyte population characterized by inflammatory phenotype: this population, although part of the leukemic bulk, lack leukemia-initiating capacity but correlated with worse prognosis in acute myeloid leukemia (AML)[85].

Caiado et al. generated a chimeric *Tet*$^{+/-}$ mouse model of clonal hemopoiesis. The clone, over time, significantly expanded compared with the *wt* counterpart; analyzing the monthly expansion rates, the authors observed that the clone expansion occurred only in advanced age (from 7 months onward) and quantifying the expression profile of key proinflammatory cytokines genes, detected an increase in IL-1α/β and *TNF-α* gene expression levels in *Tet2*$^{+/-}$ total BM cells compared with *wt.* To explore the causality link between IL-1α and clone expansion, the authors evaluated the effect of a 14-days IL1α exposure in mice carrying a 10% *Tet2*$^{+/-}$ clone: interestingly a significantly expansion of the *Tet2*$^{+/-}$ clone but not of the *wt* counterpart was observed. In a similar fashion, after IL-1α exposure, a decrease of replating capacity of HSC was detected; however, *Tet2*$^{+/-}$ HSCs were more resistant to this effect and were able to form colonies up to the fourth replating cycle. Accordingly, in a subsequent experiment, the blockade of IL-1 inflammatory reduced *Tet2*$^{+/-}$ expansion[86].

Cai et al. analyzed HSC compartments in in *wt* and *Tet2*^{KO} mice treated with Lipopolysaccharide (LPS), a ligand stimulating Toll-like receptor 4 (TLR4) and nuclear factor κB (NF-κB) signaling pathway: in both models, BM cellularity dropped. However, in *Tet2*^{KO} mice, compared to *wt* counterpart, a significant increase in pluripotent and commissioned progenitors was observed; furthermore, *Tet2*^{KO} cells showed a decreased expression of pro-apoptotic genes, including and *Bcl2l11,* with overexpression of pro-survival genes including *Bcl2. Tet2*^{KO} mice presented also an increased level of inflammatory cytokines, such as IL-6, CCL2, CCL4 TNF-α, and CXCL9; in this model, the blockade of IL-6 inflammatory signaling repressed the aberrant hematologic expansion[87].

In a similar fashion, Abeguende et al. demonstrated an advantage for *Tet2*^{ko} murine and *TET2*^{Mut} human HSPCs in an in vitro environment that contains the proinflammatory cytokine tumor TNFα. The phenotype emerged on chronic TNFα exposure was characterized by myeloid skewing and resistance to apoptosis[88].

Taken together, these evidence highlights that in a context of aging/inflammation *TET2* mutation offer a survival advantage. Furthermore, the expanded clone presents an inflammatory phenotype, perpetuating the selection process. The path to the development of malignancies can occur through an expansion of the clone and/or the cooperation with additional hyperproliferative somatic lesions.

TET2 in Hematologic malignancies

In a clinical perspective, *TET2* mutation is detected in broad landscape in myeloid and lymphoid malignancies (Figure 3).

The myeloid malignancy most commonly characterized by this mutation, as also suggested by the frequent monocytic differentiation skewing detected in the above-mentioned mice

models, is the chronic myelomonocytic leukemia (CMML), where *TET2* mutation is detected in about half of the cases (40-60%)[89-92]. The prognostic impact on these patients is still object of debate: Itzykson et al. didn't detect an independent impact in overall survival (OS)[93], whereas Coltro et al. found a survival advantage in *TET2* mutated patients and Patnaik et al. a favorable impact in *ASXL1-wt* patients[94,95]. Finally, Kosmider et al. observed a negative impact on survival[96].

The prevalence of *TET2* mutation in MDS range between 13% and 44%[69] whereas, in AML, between 8% and 30%[97-99]. A metanalysis on 1500 $TET2^{Mut}$ MDS patients highlighted no independent impact of the mutation on OS[100]; on the other hand, Kosmider et al. documented a lower incidence of leukemic progression in these patients[101]. Data on prognosis in AML patients, instead, are unclear: Weissmann et al. documented a lower event-free survival (EFS) in *TET2*-mutated patients with normal karyotype when compared to *wt*[99]. Sasaki et al identified the mutation of *ASXL1, DNMT3A, JAK2, TET2,* or *TP53* as risk factor for inferior composite complete response and OS[98]. Gaidzik, analyzing a cohort of nearly 800 patients, didn't detect any impact on the response to therapy or survival[97].

A meta-analysis on *TET2* mutation in BCR-ABL negative myeloproliferative neoplasms (MPN), by Chia et al., identified an incidence of 15.5% (Polycythemia vera 16.8%, essential thrombocythemia 9.8%, myelofibrosis 15.7%)[102]. In one of the studies with largest population, by Tefferi et al., the mutation didn't affect OS or EFS[103].

Several small-size reports pointed out a high incidence of *TET2* mutations in blastic plasmacytoid dendritic cell neoplasms (BPDCN) (20-80%)[104-107]. The small amount data doesn't allow to draw conclusion on the prognostic influence.

The impact of *TET2* mutation in lymphoid malignancies is less investigated: it's reported an incidence ranging between 4 and 30%, higher in T-cell malignancies[108-110]. Shimoda et al., analyzing a cohort of T-cell acute leukemia/lymphoma patients (TALL), didn't detect differences in OS[108].

TET2-driven CHIP and malignant progression, culprit or bystander?

This review offers the possibility to reflect more deeply on the mechanistic aspects of *TET2*-driven malignant progression. Basing on the evidence presented until this point, built on preclinical studies and animal models, since almost 10 years have passed since the first large-scale CHIP study[4], one could expect some "direct" evidence of *TET2*-driven CHIP contribution to the progression toward myeloid disease through serial measurement over time, leading from CHIP to overt MN.

However, none of the patients developing MN in the case series of Genovese et al. carried *TET2*-driven CHIP at the time of first analysis[4] and, to the best of our knowledge, clinical studies focusing on two-time points analysis on progression of the mutation are lacking. Thus, without clinical evidence of the leukemogenesis process, one could provocatively query the actual direct contribution of *TET2*-driven CHIP to the onset of the HM.

The connection between aging, inflammation and MDS is well known[111]. In this perspective, could *TET2* mutation be the epicenter of the vicious cycle leading to MN representing, thus, only an indirect epiphenomenon of the malignant progression? Or the very *TET2*-mutated clone is the direct responsible for the leukemogenesis progression through expansion and/or acquisition of new mutations (Figure 4)?

In one hand, through the above-mentioned preclinical models and single-cell analysis studies[112], it's possible to define certainty the role of *TET2* mutation in clonal architecture of myeloid malignancies. In a similar fashion, one could be tempted to attribute the same role also in CHIP; however, this would bring about ethical, clinical, and practical repercussion in the follow-up of these individuals and thus, it deserves deeper analysis. A recently published study pushed forward the study of hierarchical role of *TET2* mutation in myeloid malignancy, showing, through single cell techniques, its role in clonal cytopenia of undetermined significance (CCUS)[112]. However, although pre-malignant, this condition is ontologically different from CHIP and, again, we can't infer similar conclusion[113]. Some studies, focusing on genetic analysis of therapy related myeloid malignancies and matched pre-therapy samples, show an increasing VAF, between the two time points, of *TET2* somatic mutations. However, these observations are rare and might apply only on post-cytotoxic myeloid neoplasias, not allowing definitive conclusions on CHIP-driven malignant progression[114,115].

These speculations point out the need for more prospective studies in order to clarify the actual role of *TET2*-driven CHIP in malignant progression and to shade light on the very early (pre−) leukemogenesis.

TET2 pathway, a feasible target?

The in-deep study of biology and functions of TET2 led to several attempts of developing a target therapy for $TET2^{Mut}$ malignancies (Figure 5). Several reports pointed out the anti-leukemic potential of AA in TET2 HM as a co-Factor for Fe- and 2-Oxoglutarate dependent dioxygenases[116,117]. However, several clinical trials of AA including murine model suggested the complication of malignant cell termination as a result of activating TET-dioxygenase in HM. Some of the data in murine models studies indicated that AA enhances Tet-mediated 5-mc and promotes DNA demethylation with subsequent key function in hematopoietic homeostasis[21,118,119] and, in a recently published clinical trial, 1g/day AA supplement boosted DNA demethylation in *TET2* germline mutation carriers[120]. Intriguingly, Das et al. reported a case of a relapsed $TET2^{Mut}$ AML patient treated with high-dose AA who maintained remission for over two years[121]. The potential of AA to sensitize HSC to PARP inhibitors, which could represent a safe and efficient combination therapy has been proposed[122].

Nevertheless, it should be mentioned that the redox regulation function by AA could contribute to the anti-leukemic activity of the molecule independent of *TET2* status[117]. Accordingly, the addition of low dose of AA to Decitabine was able to improve OS and complete response rate in a cohort of elderly AML patients irrespective of *TET2* mutation[123].

Analyzing the issue from a different angle, the evidence that a minimum level of TET-dioxygenase activity is required for malignant cell survival, confirmed by the fact that the triple *TET1/2/3*^{ko} is lethal in mice models[124], prompted some authors to investigate a potential use of TET inhibitors, which are current under development[25]. A recent report confirm the hypothesis of targeting TET-dioxygenases in *TET2* mutant leukemia[25,28,125,126]. The most compelling case of targeting TET dioxygenase in *TET2* mutant HM comes from the fact that inhibiting TET2 in TET proficient healthy HSPCs promotes growth while restrict the growth of *TET2* mutant and *TET* deficient malignant clones as observed in Eltrombopag mediated TET-inhibition in aplastic anemia patient[126].

Conclusions

TET proteins have fundamental enzymatic and non-enzymatic functions in epigenetic regulation of haematopoiesis. Despite the overlapping and redundant roles among the three isoforms, the mutation of *TET2,* common event in elderly individuals, is correlated with a higher incidence of a broad range of hematologic and non-hematologic diseases. The common denominator of these conditions dwell in the tight and bidirectional connections between TET2 and aging/inflammation: the mutated clones expand in this context and promote a myeloid skewing towards committed myeloid cells characterized by inflammatory phenotypes, in a self-perpetuating scheme. Target therapies are currently under investigation with the goal to break through this maladaptive circle.

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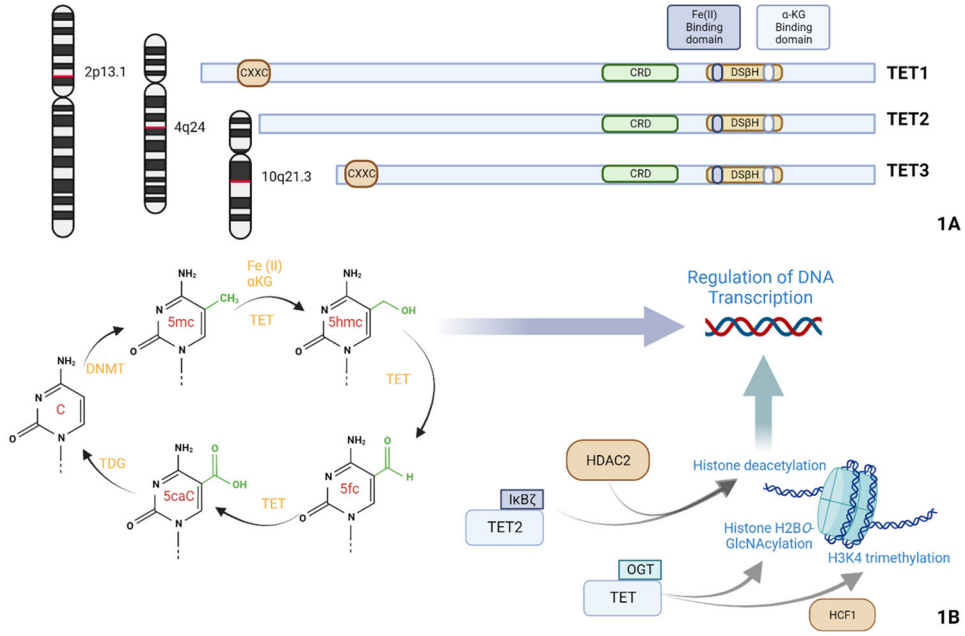

Figure 1. Schematic overview of the structure and function of TET proteins.

A, Domain organization of TET1, TET2 and TET3 proteins along with their chromosomal location. **B**, TET mechanism of action in the context of chromatin. C: cytosine; 5caC: 5-carboxylcytosine; 5fc: 5-formylcytosine; 5hmc: 5-hydroxymethylcytosine; 5mc: 5-methylcytosine; HCF1: host cell factor C1; HDAC2: Histone deacetylase 2; IκBζ: NF-kappa-B inhibitor zeta; OGT: O-linked β-N-acetylglucosamine (O-GlcNAc) transferase.

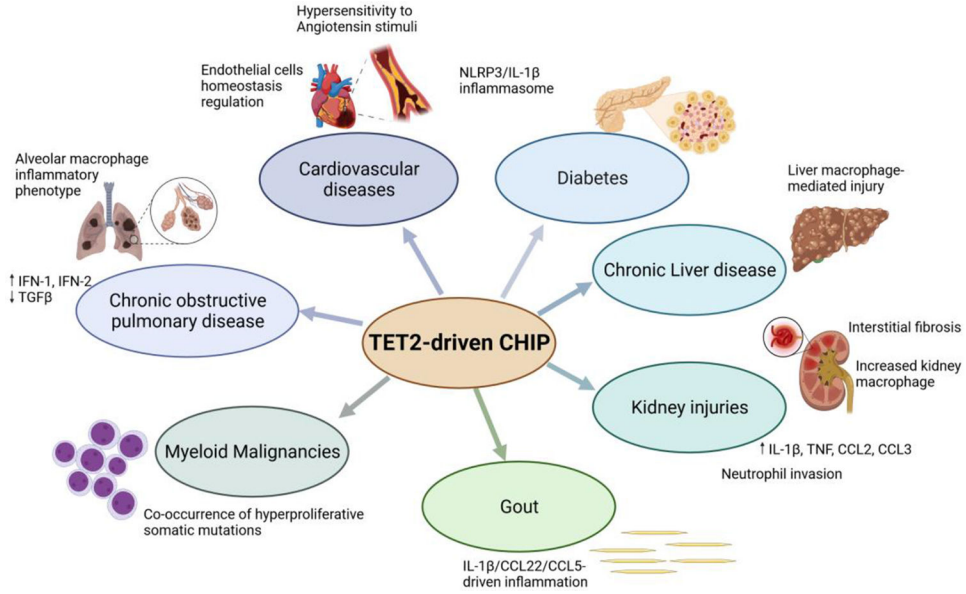

Figure 2. Schematic overview of the clinical implications of *TET2*-driven CHIP.
CHIP: Clonal haematopoiesis of indeterminate potential

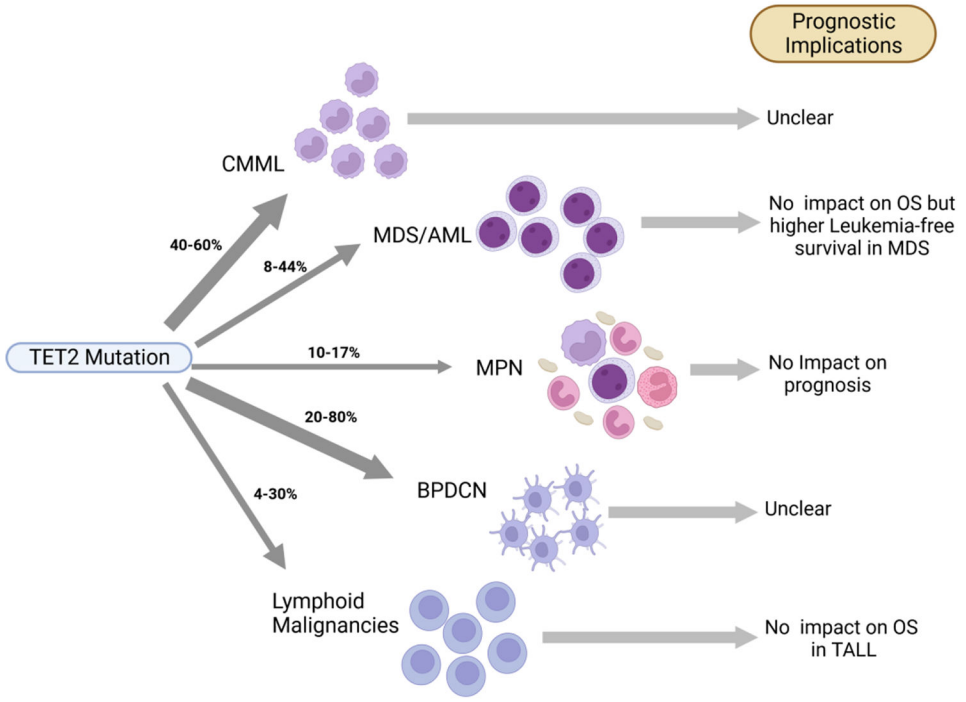

Figure 3. Incidence and impact of *TET2* mutation in Hematologic malignancies.

AML: acute myeloid leukemia; BPDCN: blastic plasmacytoid dendritic cell neoplasms; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasms; OS: overall survival; TALL: T-cell adult leukemia/lymphoma.

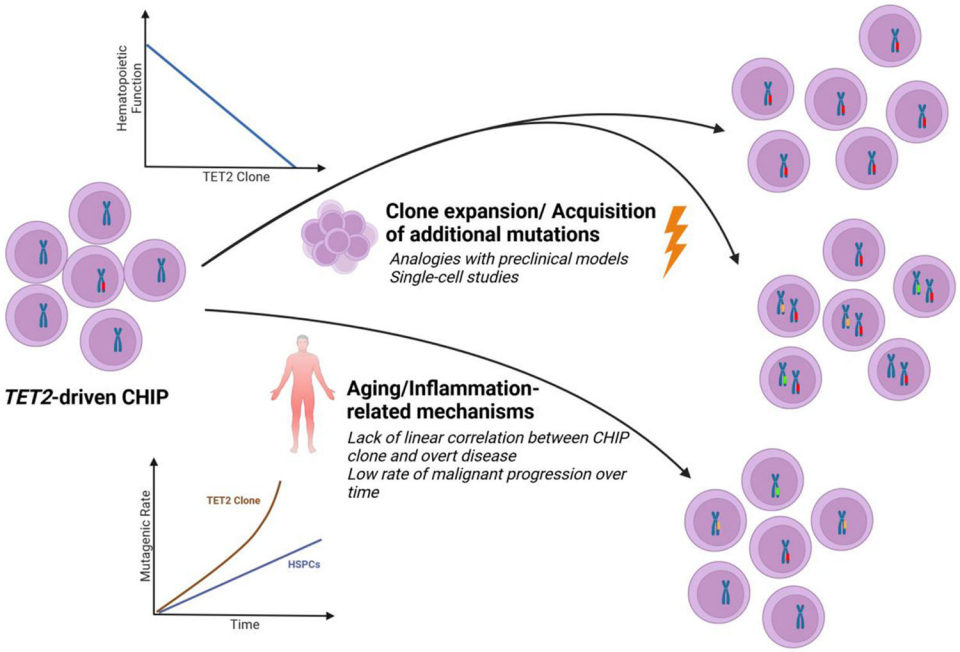

Figure 4. Possible mechanisms of *TET2*-driven CHIP malignant progression.

CHIP: Clonal hematopoiesis of indeterminate potential

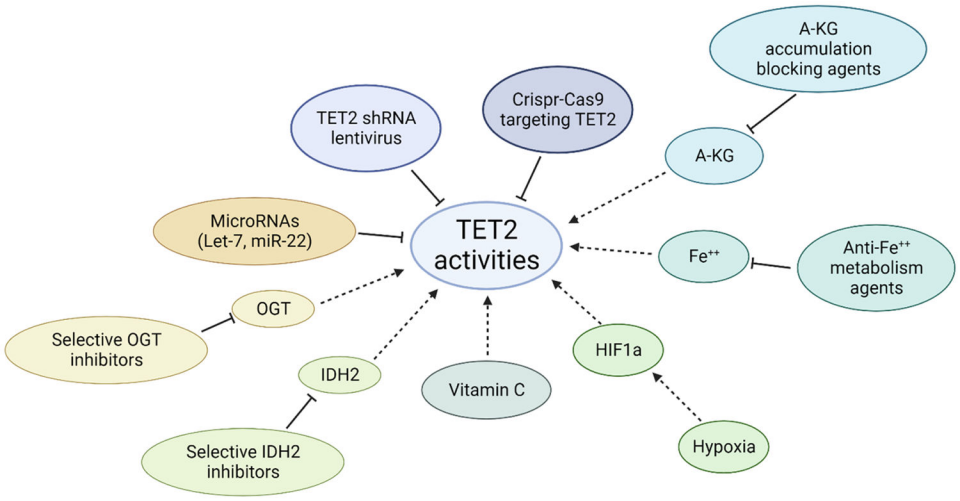

Figure 5. Molecules and compounds targeting TET2 activities.

a-KG: α-ketoglutarate; HI1F1α: Hypoxia-Inducible Factor-1 α; IDH2: Isocitrate dehydrogenase; OGT: O-linked β-N-acetylglucosamine (O-GlcNAc) transferase.