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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Methyl-CpG Binding Domain Protein MBD2 Is a Targetable Vulnerability in *TET2* Mutant Myeloid Neoplasia

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Background & Significance.Epigenetic dysregulation leading to acquired disorganization of CpG methylation (mCpG) is a hallmark of cancer in general and myeloid neoplasia (MN) in particular. TET dioxygenases (TET1, TET2 and TET3) progressively oxidize 5-methyl cytosine (mC) to facilitate CpG demethylation, a process critical to efficient transcription, lineage differentiation, and proliferation. Loss of function *TET2* mutation ($TET2^{MT}$), the predominant TET in HSPCs, isone of the most common genetic lesions in MN and often occurs early in MDS/AML as a founder lesion. It is frequently observed in clonal hematopoiesis of indeterminate potential (CHIP) [1,2], a prodromal condition linked with increased risk of MN, coronary heart disease [3], and pulmonary arterial hypertension [4]. $TET2^{MT}$ results in abnormal accumulation of mC in the genome resulting in loss of lineage plasticity due to transcriptional repression of differentiation-promoting genes and tumor suppressor genes (TSGs), contributing to a volatile pre-neoplastic state. Indeed, the accumulation of mC at otherwise unmethylated promoter- and enhancer-associated CpGs is a hallmark cancer [2,5]. The epigenetic reader mCpG binding domain protein 2 (MBD2) specifically recognizes mCpG, thereby modulating regional gene transcription [6]. This modulation occurs through the recruitment of co-repressor complexes which deliver enzymatic machinery to reorganize chromatin into compact, inaccessible, and stably silenced regions. This tightly regulated process, required for normal development and cell lineage differentiation, is hijacked by malignant cells to maintain a pro-oncogenic transcriptional program. Therefore, MBD2 inhibition may restore the normal hematopoiesis in $TET2^{MT}$ HSPCs and may represent a selective therapeutic target.

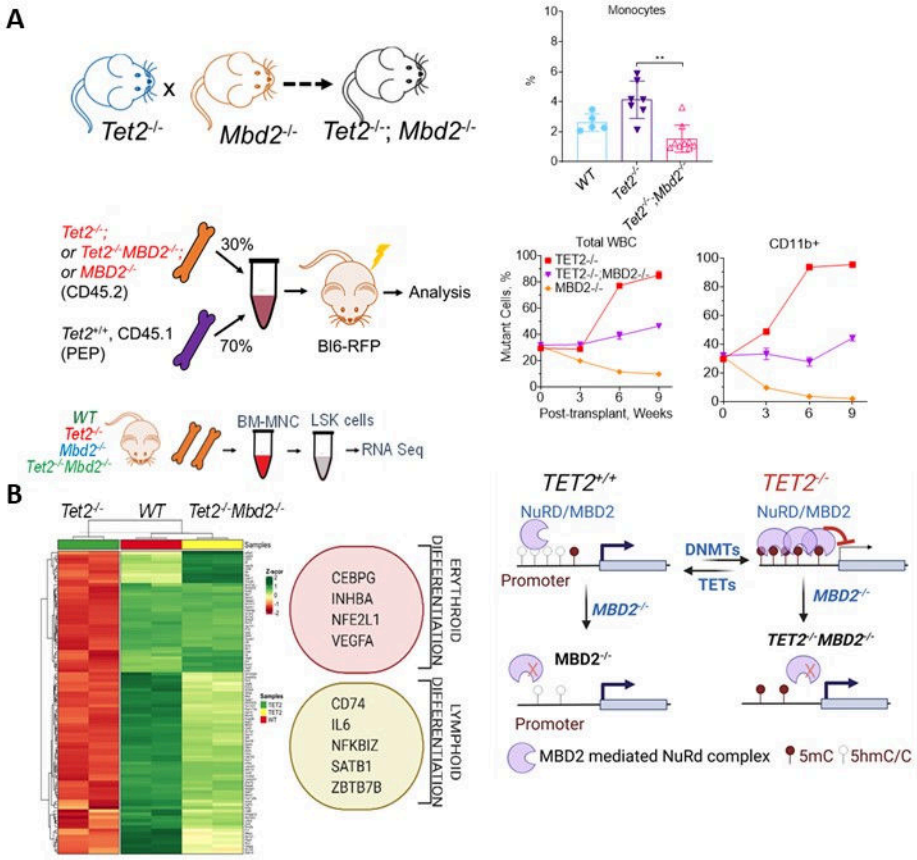
Methods.Genetic and epigenetic in vitro and in vivo model systems of clonal evolution including competitive repopulation assays and CRISPR-engineered cells coupled with NGS and biochemical analysis were used to establish the functional relevance of MBD2 in the clonal evolution of $TET2^{MT}$ HSPCs. In addition, gene knockout or knockdown (ko/kd) were used to establish MBD2 as a novel therapeutic vulnerability in $TET2^{MT}$ MN.

Results.Here we report that loss of TET activity, either by $TET2^{MT}$ or by neomorphic $IDH1/2^{MT}$, selectively enriches promoter/enhancer mCpG contributing to malignant evolution of $TET2^{MT}$ HSPCs that creates sensitivity to MBD2 inhibition. $TET2^{MT}$ creates regional accumulation of mC that recruits the MBD2-NuRd complex, resulting in silencing of TSGs as demonstrated via MBD2 chromatin immunoprecipitation assay. MBD2 ko/kd in $TET2^{MT}$ cells (SIG-M5, OCIAML-5) or isogenic CRISPR-engineered leukemic cells (THP1, K562) resulted in the termination of the malignant program as reflected in a complete loss of colony forming ability in vitro. Orthotropic transplant of SIG-M5 cells (10^5 cells/mice) created leukemia in SGM3-NSGS mice with a maximum survival of 45 days, however, $MBD2^{ko}$ in transplanted cells resulted in 100% disease-free survival of these mice. To further test if *Mbd2* loss is sufficient to restore normal hematopoiesis and if it can abrogate the proliferative advantage of $Tet2^{-/-}$ HSPCs, we developed a $Tet2^{-/-}$;$Mbd2^{-/-}$ double ko (DKO) murine model by crossing $Tet2^{-/-}$with $Mbd2^{-/-}$ mice. The DKO mice developed normally with restored hematopoiesis within the myeloid lineage compared to $Tet2^{-/-}$. Most importantly, the proliferative advantage of $Tet2^{-/-}$HSPCs was ablated, as reflected in the mutant fraction of total WBC and the restoration of $CD11b^{+}$ myeloid cells following *Mbd2* loss in competitive bone marrow repopulation assays (**Fig. A**).This restoration was mainly driven by the transcriptional rescue of differentiation-promoting genes which were silenced by promoter methylation in $Tet2^{-/-}$ HSPCs (**Fig. B**).

Discussion & Conclusion.Here, for the first time, we demonstrate that MBD2 is required for maintaining the undifferentiated state of $TET2^{MT}$ HSPCs with myeloid skewing. We provide several lines of evidence in human cells as well as in murine

models that MBD2 is a targetable vulnerability in *TET2* ^{MT}-associated leukemia. Our current findings provide a rationale for development of novel and potent non-cytotoxic therapeutic agents targeting MBD2 and their application in a biomarker-driven fashion for a large proportion of leukemias.

Disclosures Maciejewski: *Novartis:* Honoraria, Speakers Bureau; *Regeneron:* Consultancy, Honoraria; *Omeros:* Consultancy; *Alexion:* Membership on an entity's Board of Directors or advisory committees.



Loss of *Mbd2* restores normal hematopoiesis in *Tet2*$^{-/-}$ HSPCs. **A.** Generation of double knockout of Tet2 and Mbd2. The DKO mice features normal hematopoiesis in all compartments, including monocytes, while *Tet2*$^{-/-}$ demonstrates myeloid bias and results in loss of proliferative advantage of *Tet2*$^{-/-}$ bone marrow cells in a competitive transplant experiment by restoring the expression of differentiating promoting genes. **B.** Loss of Mbd2 leads to transcriptional activation of repressed gene in Tet2$^{-/-}$ HSPCs.

Figure 1

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