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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Clonal Medicine Targeting DNA Damage Response Eradicates AML

Monika Toma, PhD[1], *Adam Karami*[1], *Margaret Nieborowska-Skorska, MD PhD*[1], *Kumaraswamy Chitrala, PhD*[1], *Peter Valent, MD*[2], *Tomasz Skorski, MD PhD*[1]

[1] Fels Cancer Institute for Personalized Medicine, Temple University School of Medicine, Philadelphia, PA
[2] Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria

Clonal diversity of acute myeloid leukemia (AML) plays a key role in poor therapeutic outcomes. AML cells usually accumulate spontaneous DNA damage including highly toxic DNA double-strand breaks (DSBs) induced by metabolic products and replication stress. To repair numerous DSBs and survive, AML cells activate the DNA damage response (DDR) involving the pathways that sense (ATM and ATR kinases) and repair (RAD51-mediated homologous recombination = HR, RAD52-mediated transcription associated homologous recombination = TA-HR and single strand annealing = SSA, DNA-PK -mediated non-homologous end-joining = NHEJ, PARP1/Polq-dependent microhomology-mediated end-joining = MMEJ) DSBs. Thus, DDR is a legitimate therapeutic target.

Numerous AML-driving mutations [*FLT3(ITD), TETmut, DNMT3Amut, AML1-ETO, PML-RARa , IDH1mut, cKITmut*] regulate DDR and affect the sensitivity of leukemia cells to DDR inhibitors (DDRi). The genetic landscape of malignant clones in a patient may be complicated since individual clones can carry multiple mutations. Thus, individual AML clones may respond differently to DDRi depending on their mutational profile.

In our experimental protocol AML cells from individual patients were treated with various DDRi followed by single-cell targeted DNA sequencing (sctDNA-seq) to integrate patient's leukemia clonal composition with response to the inhibitors. We developed a sctDNA-seq myeloid platform interrogating up to 1394 genetic variants of 54 known leukemia driver genes, which unraveled the clonal landscape of AML at a single-cell resolution before and after the treatment. Based on these results we designed a "clonal attack", a patient-tailored combination of DDRi targeting all AML clones in a patient sample.

Here we show that DDRi simultaneously attacking different clones caused clonal attrition and eradicated the disease *in vitro* and *in vivo*. For example, in AML-MD2 sample 2 clones carrying *EZH2(V679M) + TET2(L1721W)* and *EZH2(V679M) + TET2(L1721W) + FLT3(D835Y)* were resistant/less responsive to ATRi but sensitive to PARPi, ATMi and RAD52i. Conversely, three other clones carrying *EZH2(V679M) + TET2(L1721W) + RUNX1(D160Y)*, *EZH2(V679M) + TET2(L1721W) + RUNX1(D160Y) + EZH2(E54*)* and *EZH2(V679M) + TET2(L1721W) + RUNX1(D160Y)EZH2(E54*) + BCOR1(R1334Tfs*32) + NRAS(G13R)* were sensitive to ATRi while less responsive to PARPi, ATMi and RAD52i.

Based on this observation, we hypothesized that simultaneous treatment with ATRi + PARPi or ATRi + ATMi should result in elimination of all AML-MD2 clones. In agreement with this hypothesis, *in vitro* treatment with these combinations was 20 - 30x more effective in eliminating clonogenic growth of Lin-CD34+ AML-MD2 cells when compared to individual inhibitors. This effect was associated with abundant accumulation of DSBs. Combinations of these inhibitors were only modestly toxic to normal hematopoietic cells. Moreover, combination of DDRi displaying similar clonal targeting specificity was only 2x better than individual inhibitors.

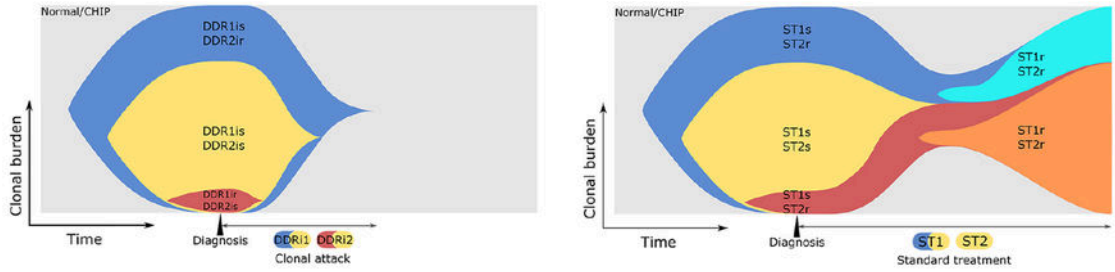
Next, humanized NRGS immunodeficient mice bearing primary AML-MD2 xenograft were treated with vehicle, PARPi, ATRi and the combination of these drugs. We found that individual treatments reduced the number of hCD45+ AML-MD2 cells in bone marrow and spleen by approximately 2x and 3x, respectively. Remarkably, the combination of PARPi + ATRi eliminated more than 99% of leukemia cells in bone marrows of 7/10 mice and in spleens of 10/10 mice while no obvious toxicity was observed.

Clonal targeting by DDR inhibitors, however, may not be applicable to all AML samples. For example, we identified AML patient samples, where clones displayed similar sensitivity pattern or were resistant to DDRi treatment.

In conclusion, we postulate that sctDNA-seq combined with *in vitro* DDRi sensitivity testing (sctDNA-seq/DDRi) is a powerful tool to interrogate clonal sensitivity of AML to these agents. This clonal medicine approach may become a novel therapeutic

regimen to overcome clonal complexity of AML in a cohort of patients. The "clonal attack" by DDR inhibitors shifts the paradigm of genotoxic therapies from those using non-discriminative cytotoxic drugs to those selectively attacking DDR vulnerabilities in AML clones with minimal harm to normal cells. Since clonal heterogeneity and DNA damage are hallmarks of cancer, the "clonal attack" may be broadly applicable to the quest for cancer cure.

Disclosures No relevant conflicts of interest to declare.



LEFT - "Clonal attack" by DDR1 inhibitor + DDR2 inhibitor. RIGHT - Standard treatment (ST) with ST1 drug + ST2 drug. Normal/CHIP = normal hematopoiesis/clonal hematopoiesis of indeterminate potential.

Figure 1

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