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ORAL ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Clonal Targeting of DNA Damage Response Pathways Eradicates Myeloproliferative Neoplasms

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Clonal diversity of myeloproliferative neoplasms (MPN) plays a key role in poor therapeutic outcomes. MPN 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, MPN 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 MPN-driving mutations [*JAK2(V617F), TETmut, DNMT3Amut, IDH1mut*] 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 MPN clones may respond differently to DDRi depending on their mutational profile.

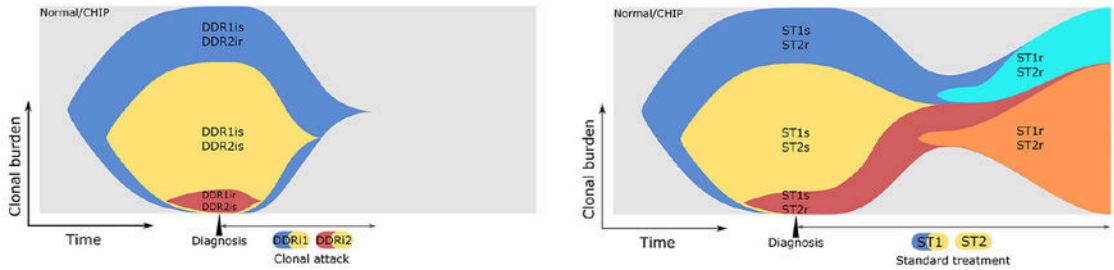
In our experimental protocol MPN cells from individual patients were treated with various DDRi followed by single-cell targeted DNA sequencing (sctDNA-seq) to integrate patient's leukemia clonal composition with response to the inhibitors. We developed a sctDNA-seq myeloid platform interrogating up to 1394 genetic variants of 54 known leukemia driver genes, which unraveled the clonal landscape of MPN 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 MPN clones in a patient sample.

Here we show that DDRi simultaneously attacking different clones caused MPN clonal attrition *in vitro*. For example, phylogenetic tree analysis of MPN patient P349 sample detected linear multi-clonal architecture with 3 Lin-CD34+ clones carrying specific sets of mutations. sctDNA-seq followed by fish plot analysis revealed clonal similarities and differences in response to DDRi. The clone carrying *KMT2A(L2373H) + SETBP1(H1100R)* was more sensitive to ATRi than RAD52i, conversely clones with *KMT2A(L2373H)* alone and *KMT2A(L2373H) + SETBP1(H1100R) + FLT3(R834L)* were more sensitive to RAD52i than ATRi. Based on this observation, we hypothesized that simultaneous treatment with ATRi+RAD52i should result in elimination of all 3 MPN P349 clones. Remarkably, the combination of RAD52i + ATRi was >100x more effective in inhibiting clonogenic growth of Lin-CD34+ P349 cells (all clonogenic cells were eradicated). On the other hand, combination of RAD52i + ATMi, the two DDR inhibitors displaying similar pattern of clonal targeting was only 2x better than individual inhibitors.

Phylogenetic tree analysis of Lin-CD34+ cells from another MPN patient P350 sample showed linear multi-clonal architecture with 4 clones. Again, sctDNA-seq followed by fish plot analysis revealed clonal similarities and differences in response to DDRi. All 4 clones displayed similar sensitivity to RAD52i and ATMi, but they responded differently to PARPi and ATRi. Clones carrying *TET2(P363L) + NRAS(G12D)* were more sensitive to ATRi than PARPi, whereas clones with *TET2(P363L) + NRAS(G12D) + DNMT3A(W330C)* and *TET2(P363L) + NRAS(G12D) + DNMT3A(W330C) + IDH2(R132C)* responded better to PARPi than ATRi. Importantly, the combination of PARPi + ATRi was >9x more effective in inhibiting clonogenic growth of Lin-CD34+ P349 cells, whereas RAD52i + ATMi was only 2x better than individual inhibitors.

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 MPN to these agents. This clonal medicine approach may become a novel therapeutic regimen to overcome clonal complexity of MPN 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 MPN 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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