Response and Resistance to Bromodomain Inhibition in Acute Myeloid Leukemia

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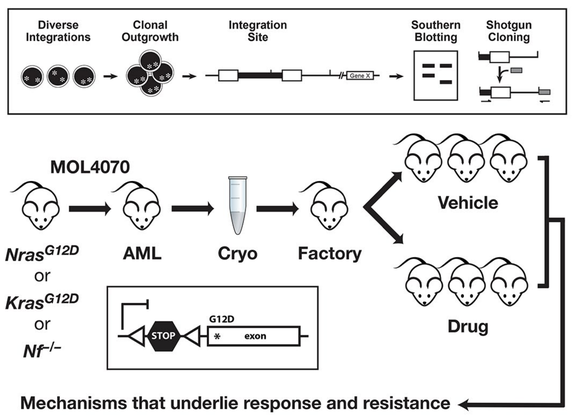
Background

Event free survival for patients with AML remains poor despite intensive myelosuppressive therapies and improvements in supportive care measures. This underscores the need for novel, biologically based therapies. Somatic mutations that deregulate epigenetic programs and signal transduction pathways frequently coexist in AML. While the former class of mutations is hypothesized to promote a chromatin state that is permissive for AML development and essential for leukemia maintenance, experimental data also suggest that signaling mutations play a central role in driving leukemic growth *in vivo*. Thus, simultaneously targeting the abnormal epigenetic landscape and aberrant signaling pathways in AML is a rational new therapeutic approach. Recent publications identified the bromodomain and extraterminal (BET) proteins, an important class of epigenetic reader proteins, as particularly promising therapeutic targets in AML. While these studies support the therapeutic potential of BET inhibition in AML, they have limitations. These include their dependence on exogenous overexpression of oncogenes, failure to inform potential combination therapeutic strategies, and a reliance on monoclonal *in vitro* systems that do not recapitulate the inherent genetic heterogeneity of human cancers.



Methods

We previously generated a heterogeneous collection of murine AMLs by infecting *Nras*, *Kras*, and *Nf1* mutant mice with the MOL4070 retrovirus, which exhibit distinct retroviral integrations that are maintained upon transplantation into sublethally irradiated recipient mice. We first established 15 mg/kg/day as the maximally tolerated dose of PLX51107, a selective and potent BET inhibitor, in sublethally irradiated mice in a C57Bl/6 x 129sv/J strain background. We performed pharmacokinetic analysis, which demonstrated excellent drug exposure at doses of 10 and 15 mg/kg/day. We next treated cohorts of recipient mice with PLX51107 and in combination with the MEK inhibitor PD0325901 (PD901). Mice that appeared ill were euthanized and underwent full pathological examination.

Figure 1: Comparative efficacy of PLX511107 and PD901 on *Nras*^{G12D} AMLs. Recipients transplanted with *Nras*^{G12D} AMLs were treated with PD901, PLX51107, or the combination (n = 5 for each treatment arm). PLX51107 dramatically extended survival. Combining PLX51107 and PD901 further improved survival.

Results

We enrolled eight AMLs, including four with a *Nras*^{G12D}, two with a *Kras*^{G12D}, and two with *Nf1* inactivation. Recipient mice received 450 cGy of sublethal irradiation followed by 2 x 10^6 leukemia cells via tail vein injection. Recipient mice were randomized to receive vehicle, PLX51107, or PLX51107 + PD901. PLX51107 markedly extended the survival of recipients transplanted with *Nras*^{G12D} AMLs that was further enhanced by PD901 (**Fig. 1**). Surprisingly, the response to PLX51107 was blunted in *Kras*^{G12D} and *Nf1* inactivated AMLs compared to *Nras*^{G12D} AMLs (**Fig. 2**). The observation of novel MOL4070 integration sites in relapsed AMLs provided definitive evidence of clonal evolution (**Fig. 3**). Importantly, we went on to show that drug-treated clones emerging at relapse demonstrate intrinsic drug resistance by re-transplanting these leukemias into secondary recipients and re-treating them *in vivo* (**Fig. 4**). Candidate resistance genes were identified by cloning retroviral integration sites. Notably, integrations near *Dnmt3b* and microRNAs were enriched in drug-treated *Nras*^{G12D} AMLs 6695 and 6606 compared to vehicle-treated counterparts (data not shown).

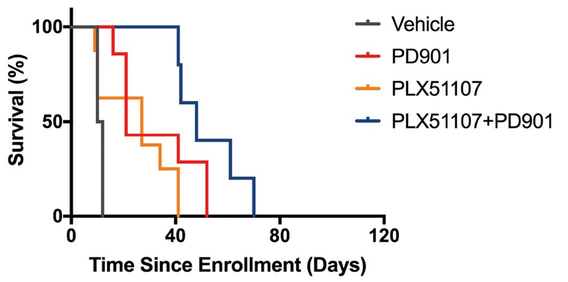


Figure 2: Differential response between *Nras*^{G12D} and *Kras*^{G12D}/*Nf1*$^{flox/flox}$ AMLs. Recipients transplanted with *Nras*^{G12D} AMLs (Fig. 1) displayed a significant improvement in median survival compared to *Kras*^{G12D} AMLs (above) and *Nf1* inactivated AMLs (data not shown).

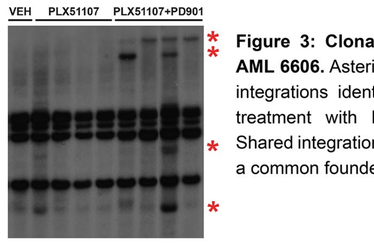


Figure 3: Clonal evolution of *Nras*^{G12D} AML 6606. Asterisks mark novel retroviral integrations identified in AML 6606 after treatment with PLX51107 and PD901. Shared integrations indicate evolution from a common founder clone.

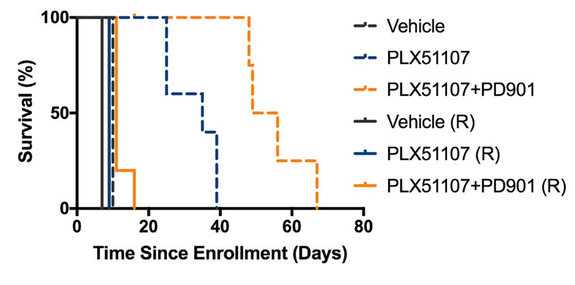


Figure 4: Intrinsic drug resistance of *Nras*^{G12D} AML 6695 after treatment with PLX51107 and PD901. AML cells isolated from mice treated with PLX51107 and PD901 were transplanted into secondary recipients and re-treated. Solid lines indicate survival of secondary recipients and dotted lines indicate survival of the initial (primary) recipients.

Conclusions

PLX51107 shows impressive efficacy in a panel of primary AMLs treated *in vivo* that is further enhanced by PD901. The differential response between *Nras*^{G12D} and *Kras*^{G12D}/*Nf* $^{flox/flox}$ AMLs leads to the intriguing and unexpected hypothesis that the type of hyperactive Ras signaling mutation may influence the response to BET inhibition in AML. We are interrogating relapsed AMLs to identify and functionally validate candidate mechanisms underlying drug resistance through the use of established strategies to directly compare vehicle and drug-treated leukemias. Ongoing studies include retroviral integration analysis, whole exome sequencing, RNA-seq, and ChIP-seq.

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Gideon Bollag and Ben Powell are the CEO and an employee of Plexxikon Inc., respectively. Please contact Ben Huang (Ben.Huang@ucsf.edu) or Kevin Shannon (Kevin.Shannon@ucsf.edu) for questions or inquiries.