Title

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Journal

Blood, 138(Supplement 1)

ISSN

0006-4971

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Publication Date

2021-11-05

DOI

10.1182/blood-2021-154233

Blood 138 (2021) 3321–3322

63rd ASH Annual Meeting Abstracts

POSTER ABSTRACTS

603.ONCOGENES AND TUMOR SUPPRESSORS: BASIC

ADAR1 Splicing Modulation As a Mechanism to Eradicate Immunologically Silent Leukemia Stem Cells

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Abstract Secondary acute myeloid leukemia (sAML) is the most therapeutically recalcitrant form of AML with a life expectancy of less than 12 months. Secondary AML evolves from relatively prevalent myeloproliferative neoplasms (MPNs), myelodysplastic syndrome (MDS), or after chemotherapy, radiation therapy, or hematopoietic cell transplantation (HCT) that together confer a 14% risk of sAML at 15 years. Cumulative sequencing studies show that human splicing factor mutations, epigenetic spliceosome deregulation, RNA editing-induced splicing alterations, and pro-survival splice isoform switching drive dormant leukemia stem cell (LSC) generation and sAML resistance to chemotherapy and molecularly targeted agents resulting in high rates of relapse. LSC are immunologically silent in part because they activate adenosine deaminase acting on dsRNA (ADAR1), which attenuates the innate immune response. In addition, therapeutic splicing modulation has the potential to induce neoepitope formation and augment checkpoint inhibitor therapy. Thus, there is a pressing need for clinical development of splicing modulatory agents that eradicate therapy resistant LSC and reduce sAML drug resistance and relapse. Rebecsinib (17 *S*-FD-895) is a pharmacologically stable, potent, and selective small molecule splicing modulator that targets the SF3B core of the spliceosome at the interface of SF3B1, SF3B3 and PHF5A. We previously showed that Rebecsinib inhibits human LSC maintenance in sAML models at doses that spare normal hematopoietic stem and progenitor cells (HSPCs). In IND-enabling studies, we now demonstrate that splicing modulation with this potent agent is a pre-clinical tox-proven strategy to eradicate LSC with the potential to overcome immune checkpoint resistance via inhibition of ADAR1 splicing and activity. We further describe targeted LSC eradication that correlates with detection of unique intron-retained and exon-skipped transcripts that can be quantified by splice isoform-specific qRT-PCR and RNA-sequencing analyses and can be used as predictive biomarkers to monitor molecular responses to Rebecsinib treatment. Mechanistically, the therapeutic effects were accompanied by on-target splicing modulatory effects, including reductions in pro-survival MCL1L transcripts and splicing factor gene products such as SF3B1 and SF3B3, which form part of the splicing modulator binding pocket as well as alterations in self-renewal promoting ADAR1 and STAT3beta transcripts. In multi-species toxicology and pharmacokinetic/pharmacodynamic studies, Rebecsinib induced splicing modulation and was well-tolerated over a broad range of doses. Because of disrupted spliceosome function, SF3B1 overexpression and increased dependence on pro-survival splice isoform expression, Rebecsinib-mediated induction of pro-survival to pro-apoptotic splice isoform switching inhibits sAML LSC survival and self-renewal at doses that spare normal HSPCs *in vitro* and in humanized mouse models commensurate with dose-dependent changes in splicing reporter exon skipping and SF3B1, MCL1, BCL2 and CD44 isoform levels. Together, this potent and selective agent along with biomarkers of response to splicing modulation provide a sensitive method of detecting activity and mechanism of action of Rebecsinib, and demonstrate its LSC selectivity in humanized stromal co-cultures and humanized mouse models, which will have utility in future clinical development of this novel therapeutic agent.

Disclosures Crews: *Ionis Pharmaceuticals:* Research Funding. **Burkart:** *Algenesis:* Other: Co-founder. **Jamieson:** *Forty Seven Inc.:* Patents & Royalties.

https://doi.org/10.1182/blood-2021-154233