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Blood 142 (2023) 1424

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Restoration of Mir-185 in Combination with BCR-ABL Downregulation By Therapeutic Delivery of siRNA with Lipid Nanoparticle Carriers Targets Drug Resistant Leukemic Stem/Progenitor Cells

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Background: Despite development of effective tyrosine kinase inhibitors (TKIs) for chronic myeloid leukemia (CML), TKI therapy is not curative as some patients develop drug resistance or are at risk of disease relapse. Importantly, CML leukemic stem cells (LSCs) remain largely unresponsive and represent a key population for sustaining disease and relapse. We previously demonstrated that miR-185 levels were significantly reduced in CD34 $^{+}$ stem/progenitor cells from TKI-nonresponders compared to TKI responders. Further, restoration of miR-185 levels in CML LSCs and their progenitors impaired their survival and sensitized them to TKI *in vitro* and *in vivo*. However, the therapeutic potential of downregulating BCR-ABL using siRNA in combination with miR-185 mimic treatment to target CML LSCs have yet to be elucidated.
Objective: Downregulation of BCR-ABL using siRNA represents a promising therapeutic approach to inhibit its kinase and non-kinase functions to combat drug-resistant CML and overcome limitations of current therapy. Additionally, previous work suggests that restoring miR-185 levels may improve therapeutic outcomes from complimentary therapies in CML, particularly in combination with BCR-ABL inhibition. Therefore, we aim to investigate the therapeutic outcomes and mechanism of action of inhibiting BCR-ABL using siRNA in combination with miR-185 mimic treatment in drug-resistant CML.
Methods: To assess the effects of BCR-ABL downregulation in combination with miR-185 restoration, we encapsulated siRNA targeting BCR-ABL and a miR-185 mimic using an optimized lipid nanoparticle (LNP) formulation. Biological effects and mechanistic changes of this novel RNA combination was evaluated using various CML cell lines expressing b2a2, b3a2, T315I mutated BCR-ABL and CD34 $^{+}$ stem/progenitor cells from non-responder patients.
Results: A total of 7 siRNA sequences targeting various fusion regions of BCR-ABL were designed and tested to effectively target primitive CML cells. The highest performing BCR-ABL siRNA encapsulated showed significant knockdown in various CML models expressing b2a2, b3a2 and T315I mutated *BCR-ABL* (65-99% knockdown). After 48 hours of 0.5 μg/mL BCR-ABL siRNA LNP treatment, BCR-ABL protein levels and its direct downstream target p-CrkL are undetectable by immunoblotting. The robust downregulation of BCR-ABL results in a dose-dependent decrease in cell viability (down to 28% and 9.4% viable cells at a dose of 1 μg/mL) and increase in apoptotic cells (70% and 78% Annexin-V positive) in imatinib resistant K562 and T315I mutated cells, respectively after 72 hours. Similarly, encapsulated miR-185 mimic downregulates its main downstream target *PAK6* transcript levels down to 50% in drug sensitive and drug-resistant cell lines and in TKI nonresponder CD34 $^{+}$ stem/progenitor cells. More importantly, our RNA LNP formulation demonstrated significant uptake (> 80% Cy5 positive) of fluorescent-labelled siRNA in TKI nonresponder CD34 $^{+}$ stem/progenitor cells and decreased *BCR-ABL* mRNA transcript levels by 60%. The combined BCR-ABL inhibition and miR-185 mimic treatment were determined to be highly synergistic when analysed using the Chou-Talalay (combination index less than 1), ZIP, Bliss and HSA methods (synergy scores larger than 10) in both drug-sensitive and drug-resistant CML cells. Furthermore, treatment of BCR-ABL siRNA and miR-185 using LNPs decreased the colony forming units of nonresponder patient cells by 50%. Interestingly, this effect was further enhanced when combined with TKIs, decreasing colony forming units to 20% compared to control after 14 days.
Conclusion: We have uncovered a LNP formulation that is able to effectively deliver therapeutic BCR-ABL siRNA and miR-185 mimic into primitive CML cells. This combination treatment significantly reduces the levels of oncogenic BCR-ABL and PAK6, decreasing the viability clonogenic potential and increases apoptosis in CML cell line models and primary patient cells. Importantly, this combination strategy overcomes TKI resistance in CD34 $^{+}$ stem/progenitor cells suggesting a feasible and effective model for patients suffering from clinical resistance and relapse.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-175044