



Splitting the message: targeting leukaemia cells with split-mRNA lipid nanoparticles

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Background: mRNA therapeutics have the potential to redefine cancer therapy. Hematopoietic stem and progenitor cells (HSPCs) are an attractive therapeutic target because they generate all immune lineages and contribute to numerous haematological diseases. In acute myeloid leukaemia (AML), malignant HSPCs sustain leukaemic stem cells (LSCs) that drive disease initiation, progression, and relapse. However, developing HSPC-targeted therapies remains challenging as they lack singular surface identifiers, yet precise payload delivery is crucial to avoid systemic toxicity. Here, we present an innovative lipid nanoparticle (LNP) platform that achieves enhanced specificity by combining antibody-mediated targeting with a split-mRNA system. Antibody-targeted LNPs deliver split-mRNAs encoding inactive protein subunits, each targeted to a different receptor. Only cells co-expressing all target receptors receive all subunits leading to protein activity. We employed this system to deliver pro-apoptotic mRNA to selectively kill LSCs. **Method:** LNPs were formulated by microfluidic mixing and functionalized with anti-Fc nanobodies that capture targeting antibodies in an optimal orientation on LNPs. Antibody-to-nanobody ratio optimization strongly impacted delivery efficiency and was essential for maximizing cell transfection. Using mStayGold mRNA as reporter, we screened LNPs functionalized with antibodies against CD11b, Ly6G, and stem cell markers CD34 and CD117, in MLL-AF9 AML cells. **Results:** CD11b- and CD117-targeted LNPs achieved the highest mRNA delivery, with >90% StayGold+ AML cells compared to 90% MLL-AF9 killing while exhibiting reduced toxicity in healthy bone marrow. By comparison, full-length DTA mRNA delivered through either CD11b- or CD117- targeted LNPs depleted entire healthy bone marrow populations, highlighting the necessity of dual-targeting. Additionally, split-DTA selectively depleted targeted T-cells in vivo, providing in vivo proof-of-concept for the platform. **Conclusion:** Our versatile platform enables precise therapeutic targeting that will advance future cancer therapies.