Combination Nanomedicines for Acute Leukemia

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Statement of Purpose: Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) collectively account for one third of cancer diagnoses and 21 percent of all cancer deaths among children and adolescents. Advances in patient risk-stratification and dose-intensification strategies for multiagent chemotherapy have led to recent improvements in overall survival for these diseases; however, a significant fraction of pediatric patients do not respond to frontline chemotherapy or later relapse with recurrent disease. One in five pediatric patients with T cell ALL (T-ALL) and one in four pediatric patients with AML die within 5 years of initial diagnosis and the treatment of adults with these diseases is even more challenging. The development of effective treatment options for patients with relapsed or refractory (R/R) T-ALL and AML thus represents an urgent and unmet clinical need.

Methods: Guided by prior reports indicating the potential for synergy between MERTK inhibition and (i) leukemia chemotherapy or (ii) antiapoptotic BCL-2 protein inhibition, we developed a high-throughput combination drug screen in which we sought to identify ratiometric drug synergy between the small molecule and dual MERTK/FLT3 inhibitor, MRX-2843, and (i) methotrexate and/or vincristine, as well as (ii) venetoclax, obatoclax, or gossypol. Using a high-throughput liquid handling robot (Beckman Coulter NX), we then constructed 3- and 2-dimensional drug interaction matrices comprised of >530 or >150 distinct ratiometric drug combinations, respectively. A diverse panel of leukemia cells (n=21) were exposed to these drug interaction matrices for 72 h (n=4 and 3 replicates, respectively) after which growth inhibition was measured via CellTiter-Glo 2.0 ($Z' \geq 0.5$) and synergy was assessed by various methods to yield matrices of ratio-dependent drug synergy, additivity, or antagonism for each cell line or, when averaged, each leukemia cell lineage.

Results: From these screens, we identified discreet "windows" of ratiometric drug synergy that were both abundant and conserved across cell lineages. Synergistic drug effects were less prominent in some instances, for example combination drug exposure in B cell lineages; however, ratiometric drug synergy was most prominent and consistently observed from (i) the combination of MRX-2843 and vincristine in T-ALL (18-71 mol:mol ratio) and (ii) the combination of MRX-2843 and venetoclax in AML (0.001-15 mol:mol ratio). Having identified and validated novel pairwise drug combinations specifically tailored to T-ALL or AML, we next developed drug formulations to conditionally maintain ratiometric drug synergy – despite substantial differences in the *in vivo* disposition of these molecules – by encapsulating these compounds within 80 nm lipid vesicles comprised of DSPC, DSPG, and cholesterol (7:2:1 mol) and loaded via the pH gradient method. We them validated that these formulations maintained ratiometric drug synergy *in vitro* following the treatment of T-ALL (Jurkat) and AML (OCI-AML-5) cells multi- and single- agent liposomes, respectively, as measured by LC-MS/MS of cell lysates.

To study the therapeutic potential of nanometer-scale drug formulations with T-ALL-tailored drug synergy, we performed dose-finding studies in NRG mice and identified maximum tolerable doses (MTDs) for single-agent and multi-agent drug formulations containing synergistic, antagonistic, and additive drug ratios as defined in prior drug screens. We then studied the efficacy of these formulations at MTD-dosing levels (1.2-31.8 mg/kg total drug, i.p. qw x 4w) in NRG mice bearing human T-ALL (Jurkat-Luc) xenografts (n=5). Strikingly, we observed trends in treatment response (bioluminescence) and overall survival confirmed results of the prior drug screens: synergistic>additive>>> antagonistic formulations. We further examined the efficacy of conditionally synergistic nanoparticles containing MRX-2843 and vincristine in NRG mice bearing high (~100x) T-ALL disease burden (n=6) and observed substantial improvements in overall survival relative to vehicle nanoparticles (49d v 25d median).

Conclusions: Together, these data show that high-throughput combination drug screening and formulation can be used to identify and conditionally maintain ratiometric drug synergy, respectively, *in vitro* and *in vivo*. Specifically, we show that MRX-2843 synergizes with (i) vincristine chemotherapy in T-ALL and (ii) with venetoclax BCL-2 inhibition in AML, and that single- and multi- agent combination drug formulations can recapitulate this ratiometric drug synergy *in vivo* to improve treatment outcomes in mouse models of T-ALL. While efficacy studies in AML are ongoing, given the modular nature of this approach to combination drug discovery and formulation, we anticipate rapid extension of this methodology to other drug combinations and diseases in the future, including solid tumors and non-malignant diseases

References: n/a (unpublished)