Streamlining preclinical in vivo treatment trials by multiplexing genetically labelled PDX models of several patients in a single mouse

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Introduction:

Better treatment options are intensively needed for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Before application in clinical trials, novel therapies require preclinical testing which is resource intensive. In previous work, we had shown that multiplexing several PDX cell populations within a single mouse in competitive *in vivo* experiments gives identical results to studying each population in separate mice (Liu et al., Biomarker Research 2020).

Materials and Methods:

From primary patient material, we had established patient derived xenograft (PDX) mouse models of ALL and AML, which allow serial transplantation in immunodeficient NSG mice. We labelled 5 individual samples with both a unique fluorochrome and a unique genetic barcode, for later detection by flow cytometry or next generation sequencing, respectively. In addition, luciferase was recombinantly expressed in all samples to allow repetitive monitoring of leukemia growth using bioluminescence *in vivo* imaging (BLI). Up to 5 PDX samples, either ALL or AML, were multiplexed and aliquots injected into groups of mice (n=4-6). For post mortem analysis, PDX cells were reisolated from murine bone marrow and spleen and absolute tumor burden was quantified by flow cytometry. Determining fluorochrome composition allowed quantifying the proportion that each of the 5 samples contributed to the entire tumor burden.

Results and Discussion:

Mice containing multiplexed PDX cells from 5 different patients with ALL were treated for 2 weeks with either verum treatment or with solvent as control. In a study using the BCL-2 inhibitor Venetoclax in PDX ALL models, we were able to distinguish between sensitive and resistant PDX samples. While two samples showed a drastic decrease in tumor burden, three samples showed no or only a mild response. This effect could be observed both in cells isolated from bone marrow as well as spleen. Variances between mice were rather small, with few exceptions for some treated mice. With this approach, we were able to define the effect of a single drug on up to five distinct samples in parallel, reducing resources by factor 5.

Conclusion:

Taken together, we established a multiplex protocol for *in vivo* therapy trials that allows simultaneous testing of up to 5 PDX samples in competitive *in vivo* trials. The approach reduced the required number of experimental mice by a factor 5, in line with the 3R concept. In the future, our approach might rationalize *in vivo* drug trials.

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