Title: Precision oncology in childhood acute myeloid leukemia by functional genomics

Author: Wang Han, Chan Yuen Yee Kathy, Lee Po Yi Polly, Cheng Wai Tsoi Frankie, Lam Kee See Grace, Chow Tin Wai Terry, Leung Wing Kwan Alex, Li Chi Kong, Leung Kam Tong

Background/Aims: Despite advances in chemotherapy-based treatment protocols, the outcomes of children with acute myeloid leukemia (AML) remain suboptimal. Implementation of targeted therapy based solely on genomics is challenging due to the complex mutational patterns and scarcity of pharmacologic agents for most lesions. In addition, pediatric and adult AML are genetically and biologically distinct, which poses a major hurdle for extrapolation of new agents approved for adult AML to the pediatric population. This study aims to adopt a functional approach that directly measure the response of patient-derived leukemic cells to targeted agents, and to establish the drug sensitivity pattern and identify candidates of immediate clinical relevance for precision usage in high-risk pediatric AML.

Methods: A high-throughput drug screening, comprising 39 targeted agents (2 in Phase I, 10 in Phase II, 5 in Phase III, 22 FDA-approved) and 6 conventional chemotherapeutics, was performed on 30 pediatric AML samples collected at diagnosis or relapse using a serum-free, cytokine-supported culture system. A counter-screen of active drugs on cord blood hematopoietic stem cells was accomplished to reveal leukemia-selective activities. The robustness of the drug testing platform for predicting in vivo activities was validated in advanced animal models. Genomic profiling was complementarily performed to identify the genetic markers and underlying mechanisms of drug sensitivity. Patients with refractory AML were treated with targeted agents based on drug profiling results, and assessed for clinical responses.

Results: Unsupervised clustering revealed 5 distinct clusters of drug response: highly active compounds (IC50 <20nM, 6 drugs); generally active compounds (IC50 <500nM, 11 drugs); compounds with bimodal activities (wide IC50 ranges, 4 drugs); generally inactive compounds (14 drugs); and inactive compounds (10 drugs). Targeted agents, including Bcl-2, HDAC, proteasome, HSP and survivin inhibitors, had substantially higher potency and selectivity over standard chemotherapeutic agents. New agents approved for adult AML were essentially inactive in pediatric AML. Drug sensitivity ex vivo accurately predicted in vivo single-agent and combinatorial activities with cytarabine in cell line- and patient-derived xenografts. Targeted resequencing of a 141-gene panel revealed novel mutations of prognostic relevance, such as KMT2C, in pediatric AML and their vulnerability to targeted agents. Whole-genome RNA-sequencing identified distinct gene expression signatures shaping the response to individual drugs. Administration of venetoclax and dasatinib to two children with refractory AML resulted in rapid blast clearance and achieved long-term remission.

Conclusions: Our study establishes a reliable drug testing platform and a pediatric-specific drug response profile of AML, which enables an evidence-based selection of targeted agents for patients without treatment options and endows therapies increasingly precise and personalized. The study also generates a valuable gene-drug-clinical dataset that could be leveraged to address the fundamental and translational biology of pediatric AML. It will ultimately impact the future design of clinical trials and protocols for managing this life-threatening malignancy.