Drug Response Profiling informs personalized bridging to cell therapy for patients with relapsed/refractory acute lymphoblastic leukemia

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618. Acute Lymphoblastic Leukemias: Biomarkers, Molecular Markers and Minimal Residual Disease in Diagnosis and Prognosis

Introduction Treatment of refractory/relapsed acute lymphoblastic leukemia (ALL) remains challenging in spite of recent progress with immunotherapy and targeted agents, such as BH3 mimetics or tyrosine kinase inhibitors. Growing appreciation of the molecular heterogeneity of the disease with subtype-specific cancer dependencies positions functional drug testing as a promising orthogonal precision modality to improve the prediction of drug activity and adapt treatment. We leverage automated, imaging-based *ex-vivo* Drug Response Profiling (DRP) to deliver individualized therapeutic options in real time for patients with urgent medical needs. Here we report on the DRP experience of 297 primary ALL samples from 271 patients with BCP-ALL, T-ALL and mixed phenotypic acute leukemia (MPAL) registered from 2016 to 2022 in a non-interventional setting.

Methods We have generated a reference set of 137 diagnostic samples from patients with high-risk (74%) or standard/medium-risk (26%) disease as defined by the clinical trial AIEOP-BFM ALL 2017, as well as 117 and 43 samples from patients with first or more advanced relapse respectively. We assayed cell viability at a single-cell level from viably frozen samples co-cultured with hTERT-immortalized mesenchymal stromal cells (MSC) after a 72h drug incubation with a library of up to 110 compounds at five concentrations in duplicates. We assessed total cell counts by classifying MSC, live and dead leukemia cells using a supervised machine learning model deployed on cloud infrastructure. We longitudinally evaluated consistency in drug activity with PDX models and monitored integrity of the stromal layer on a per-sample basis. In a subset of cases (15-20%), relevant stromal detachment or low leukemia survival led to samples not meeting all quality control standards. Drugs were ranked by the area-under the dose response curve and referenced to all previous samples. This patient-

specific *ex vivo* drug fingerprint was reported to the treating physician with a typical turn-around time of less than two weeks.

Results Integration of *ex vivo* dose-response profiles amongst front-line patients receiving a one-week cytoreductive steroid pre-phase followed by a four-drug induction regimen revealed a correlation of Dexamethasone sensitivity with clinical responses at d8 and MRD at d15 and d33. In addition, we observed differential vulnerabilities to BCL2 and tyrosine kinase inhibitors in relevant BCP- and T-ALL subtypes.
For patients with refractory or relapsed disease, DRP was offered to support the treating physician in identifying a bridging regimen prior to subsequent consolidation with cellular therapy. We profiled 68 r/r T-ALL samples from 12 countries and found actionable drug activities across a spectrum of targeted agents including Venetoclax, Bortezomib and Selinexor as well as different purine and pyrimidine analogs. In 12 T-ALL and 5 BCP-ALL a DRP-informed regimen was implemented in real-time to bridge towards either HSCT or CAR-T (Table 1). Bridging strategies including DRP-guided treatment elements were able to stabilize or reduce disease burden in 15/17 patients allowing to transplant or infuse CAR-T cells in 12 patients with 8 patients being in remission at last follow-up.

Conclusion We describe an array of pharmacological sensitivity and resistance patterns across a heterogeneous, high-risk cohort of BCP- and T-ALL patients and report detection of individual susceptibilities to targeted agents of different classes (BH3-mimetics, proteasome inhibitors, TK inhibitors, Exportin-1 inhibitors). Systematic recording of drug sensitivity profiles offers opportunities for mechanistic explorations and informs personalized therapy decisions. We advocate that DRP is included in early phase, randomized, prospective clinical trials in the r/r ALL setting to prepare translation into future precision trials that comprehensively integrate molecular and functional information to improve treatment outcome.

Table 1. BCP- and T-ALL patients with a DRP-informed individualized bridging element to cell therapy.

LKnumber	Patient	diagnosis	Stage	Bridging intention	Treatment element	Bridge assessment	SCT post DRP / CAR-T	Final outcome
2017.097	1	T-ALL	relapsed	HSCT	Dasatinib, Cytarabine, PEG-Asparaginase	CR	no	PD, death
2021.111	2	T-ALL	de novo refractory	HSCT	Dasatinib, Venetoclax, Dexamethasone	SD/NR in BM; PR in PB	no	PD, death
2022.136	3	MPAL (T/Mye)	de novo refractory	HSCT	Ponatinib, IB *	CmR	yes	CmR
2018.039	4	T-ALL	de novo refractory	HSCT	Venetoclax, Bortezomib	PR	yes	PD, death
2019.044	5	T-ALL	relapsed	HSCT	Venetoclax, Bortezomib	CR	yes	GVHD, death
2019.045	6	T-ALL	de novo refractory	HSCT	Venetoclax, Bortezomib	CR	yes	PD, death
2019.047	7	T-ALL	relapsed	palliation	Selinexor	PR in BM; CR in PB	no	PD, death
2022.020	8	T-ALL	de novo refractory	HSCT	Venetoclax, (+FLAG/IDA)	CR	yes	CmR
2022.131	9	T-ALL	relapsed	HSCT	HC2 + Venetoclax, Bortezomib	CR	yes	CmR (3 mo)
2016.044	10	T-ALL	relapsed	HSCT	Carfilzomib	ITT	no	PD, death
2021.099	11	T-ALL	relapsed	CD7 CAR-T	Selinexor, Bortezomib, Asparaginase	ITT	no	PD, death
2022.101	12	T-ALL	relapsed	CD7 CAR-T	Venetoclax, Decitabine	SD	yes	CmR (3 mo)
2020.044	13	BCP-ALL	relapsed	CD19 CAR-T	Venetoclax, modified IB	SD	no	CmR (18 mo)
2021.042	14	BCP-ALL	relapsed	CD19 CAR-T	Bortezomib, Venetoclax, Azacitidine	SD	no	death (12 we)
2022.113	15	BCP-ALL	relapsed	CD19 CAR-T	Bortezomib, Venetoclax, Cytarabine	PR	yes	CmR (6+1 mo)
2021.102	16	BCP-ALL	de novo refractory	CD19 CAR-T	Ponatinib **	CR	yes	CmR (4+11 mo)
2021.095	17	BCP-ALL	relapsed	CD19 CAR-T	Trametinib, Cytarabine	SD	yes	CmR (3+12 mo)

PD: progressive disease (>50% relative blast increase), SD/NR: stable disease (± 50% relative blast change), PR: partial response (>50% relative blast reduction), CR: complete remission (<5% blasts), CmR: complete molecular remission (MRD<10^{-4}), ITT: intent to treat, PB: peripheral blood, BM: bone marrow.
IB: cyclophosphamide, 6-mercaptopurine, Cytarabine, HC2: HR consolidation with Dexamethasone, Cytarabine, Etoposide, PEG-Asparaginase
* Patient with NUP214-ABL fusion and Ataxia Telangiectasia. Bridging with Ponatinib to minimize toxicity.
** ABL fusion with unknown functional consequence.