Ex-Vivo Drug Response Profiling for Precision Medicine Approaches in Acute Myeloid Leukemia with the Open Microwell Microfluidic Platform

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BACKGROUND

Patient stratification to match individual patients with the most effective drug treatment is still a major open challenge in cancer care. For instance, cytarabine is the main drug used for AML treatment but 30% of patients fail to respond to this agent. Laboratory developed tests determining ex-vivo cellular response to cytotoxic anticancer drugs have demonstrated good correlations with clinical response, sometimes surpassing the predictive power of molecular and genetic profiling. Standardizing sample processing to remove operator-dependent biases and maintaining live cells in a functional status that closely resembles in-vivo function are major challenges affecting these tests. Here we present the *Open Microwell* (OMW) platform, a microfluidic-based system that integrates the entire process of ex-vivo testing of anticancer drug efficacy and enables drug testing in the clinical setting prior to therapy administration. The concept was validated for the first time on 14 AML patients at Sant'Orsola hospital, Bologna, Italy, showing the possibility to initiate the analysis readily after sample collection, thus minimizing drifts in cell function that typically start occurring within hours from sampling. Results are provided in about 24 hours, with a fully-automated system.

METHODS

2 ml of fresh bone marrow in EDTA and 1 ml of serum blood were collected from each patient. White blood cells (WBC) were separated from bone marrow by standard Ficoll-Paque, suspended in medium supplemented with 2% autologous serum and loaded in diagnostic kits integrating a microfluidic device with 16 microchannels and 1200 microwells/channel (70 µm diameter), open at the bottom end (Fig. 1 and 2A-B). After settling down in microwells, cells were stained with CMAC cell tracker, anti-human-CD34/CD45 fluorescently-labeled antibodies and Propidium Iodide (PI) by injecting the reagents in the microchannels. Cytarabine or combination therapies (FLAI-3, FLAI-5, FLA, MEC-4) were then injected in the microchannels and cells were incubated for 24 hours in the system (Fig. 2C). Four channels were used per therapeutic condition, including reference (ref), high (ref x 10) and low (ref/10) dosages plus a non-treated channel as reaction control. Finally, a custom software was used to detect cells in images, classify AML blasts and analyze cell death (Fig. 2D). Drug efficacy was determined by evaluation of both cell depletion and apoptosis induction.

Figure 1. Disposable diagnostic kit

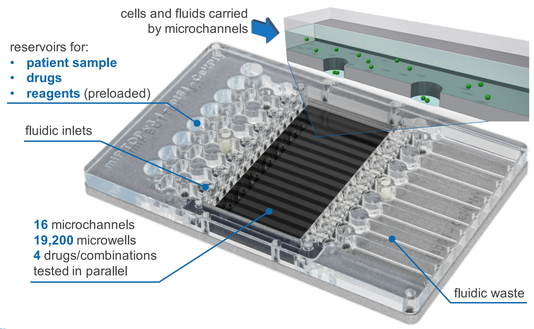
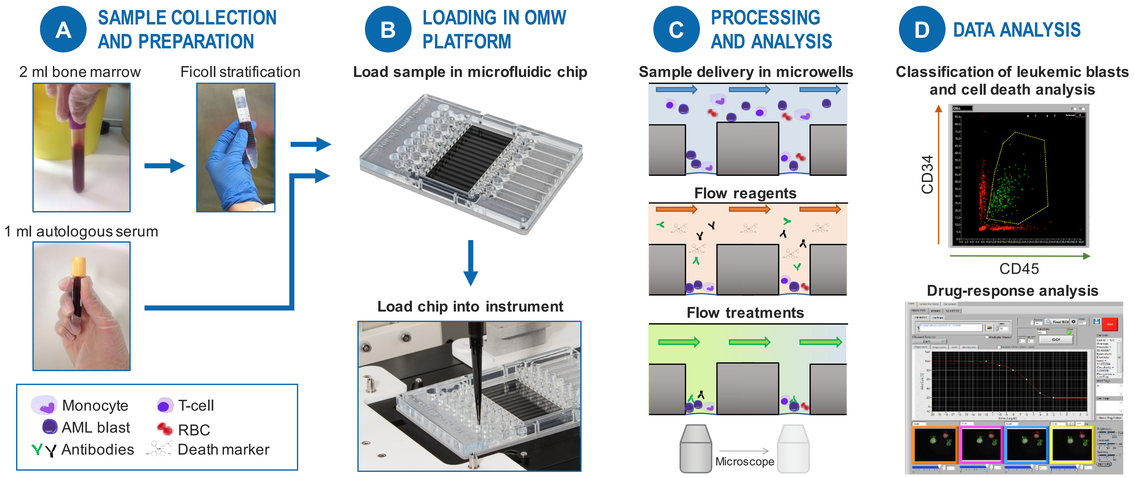
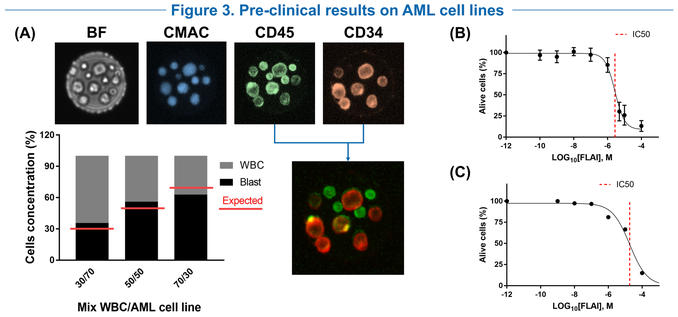


Figure 2. Workflow



PRE-CLINICAL RESULTS

Figure 3. Pre-clinical results on AML cell lines



We first validated the assay in a pre-clinical study using commercially available AML cells lines KG-1 and HL-60. WBC were separated from healthy donors blood samples by standard Ficoll-Paque. KG-1 cells were then mixed with WBC at different ratios (30:70, 50:50, 70:30). Results indicates that OMW platform can detect KG-1 cells identified by anti-human-CD34/CD45 fluorescently-labeled antibodies in a mixture with a good correspondence to the Expected result (A). The efficacy of one of drug combinations used as first line therapy in AML (FLAI) was then evaluated on HL-60 cells. A dose-response curve was built using Alamar Blue assay (B) or treating the cells inside the OMW platform (C). The results demonstrate the feasibility of the reagents selected for the assay as well as the usability of the OMW platform for such a model.

CLINICAL VALIDATION AND RESULTS

14 AML patients corresponding to the inclusion criteria indicated in Table 1 were enrolled into the clinical validation study. We first validated the OMW platform against a gold standard (FacsAria flow cytometer) for the detection of tumor cells and measurement of cell viability and apoptosis. Count of blast frequency was carried out on 5 patient samples using anti-CD34/CD45 staining. Results (Fig. 5B) show a correlation between the gold standard and the OMW. AML blast viability was carried out on 4 patient samples after 24h drug stimulation and in control situations (vehicle only). OMW and the gold standard (incubation in 96-well plates followed by flow cytometry analysis) showed the correlation reported in Fig. 5C. Then we tested the predictive power of the OMW system by comparing *ex-vivo* drug response analysis with clinical outcomes. CellPly test score was set as the ratio between the number of dead cells treated at high dosage and control. Equivalent results were obtained using the ref dosage. The test was performed as a blind prospective study for 3 patients and in a retrospective way on residual AML blasts after therapy for 2 patients. Fig. 4 reports the score obtained for the 5 patients and compares it with the clinical outcome, showing that 2 groups are identified by the test.

Table 1. Inclusion criteria

Diagnosis	• Primary Acute Myeloid Leukemia (AML) • AML from MDS
Subtype	• AML, type M0,M1,M2 with/without CD34+ immunophenotype • AML type M4-M5 with blast count >80%
Age	<65 y.o and >65 y.o.
Status	• Relapse • Refractory • De novo
Therapies	• FLAI-5 • FLAI-3 • FLA • MEC-4 • Cytarabine
Source	• Bone Marrow • Autologous serum
Time to follow up	20-45 days
Clinical follow up type	Bone marrow blast count
Endpoint	Clinical follow up: • Stable Disease (SD): refractory • Complete Remission (CR): less than 5% of immature cells in the BM

Figure 4. Patient score and clinical outcome

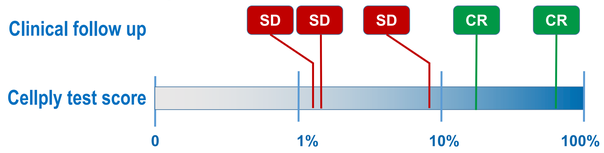
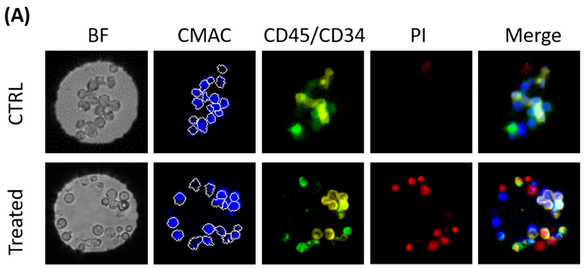
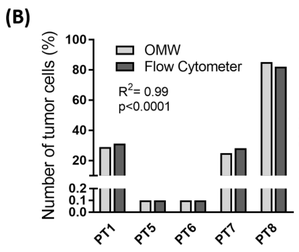


Figure 5. Results of patient sample analysis in OMW

(A) Image analysis acquisition. (B) Classification of AML blasts. (C) Identification of death AML blasts after treatment.





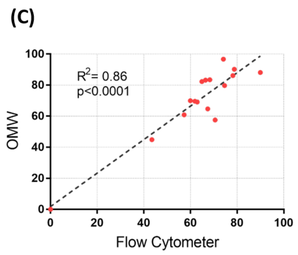


Table 2. Patient characteristics, results of the analysis and clinical outcome

Patient ID	3	12	4	13	14
Type of study	Retrospective	Retrospective	Prospective	Prospective	Prospective
Age / Sex	63 / female	67 / male	63 / female	48 / male	24 / female
Status	AML from MDS, Refractory	Relapse	AML from MDS, Refractory	De novo	De novo
Blast count	27.8%	70.2%	27.8%	46.8%	32%
Therapy	FLAI*-3	FLA**	Cytarabine	FLAI*-5	FLAI*-5
Cellply test score	9.7%	<5%	<5%	72.3%	26.68%
Follow up	SD	SD	SD	CR	CR
ELN genetic group	Intermediate-I	Intermediate-II	Intermediate-I	Adverse	Favorable

FLAI: Fludarabine + Citarabine + Idarubucine. FLA: Fludarabine + Citarabine. MEC: Mitoxantrone + Etoposide + Citarabine. ELN: European LeukemiaNet.

CONCLUSION

The OMW platform was able to count AML blasts and analyze viability and apoptosis showing high concordance with conventional diagnostics represented by flow cytometry. Comparison between test score and clinical outcome performed on 5 patients shows that 2 subgroups can be identified, each group containing responders and non-responders. By comparison, the ELN genetic group classification does not stratify patients with homogeneous clinical outcomes. The assay requires 30 µl of bone marrow sample and simple manual pre-processing. Profiling of patient response to specific drugs or combinations is provided within 24h. These features make the OMW platform suitable for near-patient analysis, to evaluate drug activity on tumor cells within a heterogeneous sample. The presented approach is promising for patient stratification and for guiding personalized treatment in hematologic malignancies, with a first proof achieved on AML patients.

CONTACT INFORMATION

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Disclosure: L. Rocchi, A. Faenza, L.Rambelli, V.Guadagnuolo, N.Pecorari, L.Giulianelli, D. Biscarini report employment for CellPly S.r.l; R. Guerrieri reports equity ownership for CellPly S.r.l.; M. Bocchi reports equity ownership and membership on an entity's board of directors or advisory committees for CellPly S.r.l.; G. Martinelli reports consultancy for MSD and Genentech; consultancy and speaker bureau for Roche, Ariad, Pfizer, Amgen and Celgene and speakers bureau for BMS and Novartis.

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