Inter-Cell Networking Profiling Enables Comprehensive Characterization of Immune-Mediated Activity of Anti-CD38 Therapy through Ex-Vivo Analysis of Multiple Myeloma Patients

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ASH 2019, Orlando, FL

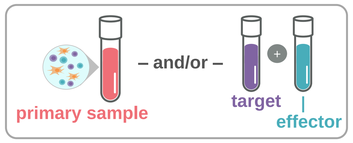
BACKGROUND

There has been a fast progress in clinical use of antibody-based immunotherapy, given the superior efficacy commonly achieved in clinic and the limited toxicity. However, personalization of treatment remains of major importance, both to achieve better clinical performance for monotherapy and to identify the best combinations on a patient-by-patient basis. Predicting patient's response to immune-oncology treatments is complex due to the need to characterize both tumor response and immunologic mechanisms possibly activated by the therapy, including antibody dependent cellular cytotoxicity (ADCC). We present the Inter-Cell Networking Profiling (ICNP), a novel analytical method enabling a comprehensive and precise characterization of the modulatory effect of immunotherapies on immune-tumor cell interactions. We validated the ICNP on multiple myeloma (MM) patient samples to characterize the efficacy of Daratumumab, an anti-CD38 antibody (Ab).

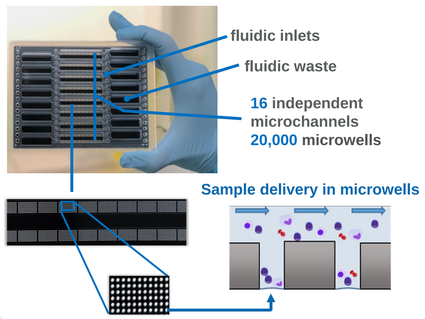
METHODS

Sample preparation

Bone marrow samples in EDTA were collected from 13 MM patients (7 de novo, 6 relapse). 8 samples were processed by Ficoll-Paque, preserving the original composition of effector (E) and target (T) cells, i.e. NK and plasma cells respectively (primary samples). 5 samples were processed to obtain co-cultures of WBC depleted of plasma cells (which include NK cells as effectors) and U-266 or NCI-H929 MM cell line (target). NK and plasma cells were stained with anti-CD16/CD56 and anti-CD138 fluorescently-labeled Abs, respectively. Propidium Iodide (PI) was used as cytotoxicity marker.



Cell delivery

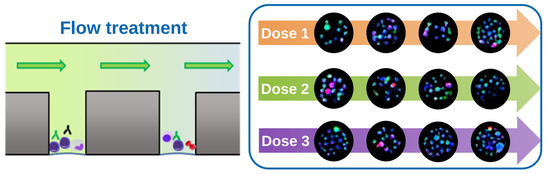


The Open Microwell (OMW) is a microfluidic device on which living cells extracted from ex-vivo patient samples are loaded through an automatic liquid handling system, forming 20,000 miniaturized heterogeneous cell clusters in microwells and allowing analysis of cell-cell interactions in each cluster under the action of specific treatments. Each of the 16 independent microchannels is used to test a different condition (e.g. a drug at a specific dose) on a group of 1,200 microwells.

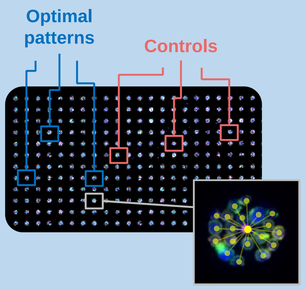
A statistical model (Figure 1) was created to define the optimal experimental setup (E:T initial ratio, cell concentration) given the composition of the biological sample (NK and plasma cells portions), in order to maximize the frequency of patterns of interest within the 20,000 microwells, such as the co-localization of E and T cells in a desired E/T co-localization factor (E/T_{CF}).

Drug delivery

System automation allows to inject drugs in the microchannels and expose cells trapped in microwells to treatments. The effect of Daratumumab at 3 different dosages (0.1 µg/mL, 1 µg/mL and 10 µg/mL) or no drug (control) was analyzed through fluorescence time lapse microscopy for up to 12 hours.



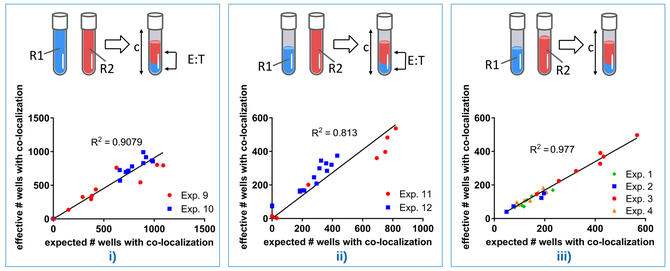
ICNP: a novel analytical method for precise characterization of drug action



ICNP is an analytical method enabled by Open Microwell allowing the analysis of patient's cells in specific groups of cell clusters selected according to common cell-cell interaction patterns. For example, cell clusters are grouped according to cell-cell proximity, cell density, immunophenotype of neighbor cells (e.g. tumor, immune cells), viability status of neighbor cells. Such information, often ignored in averaged analysis, play a key role in the evaluation of drug action on cells, including both targeted- and immune-therapies. In this work, we show how ICNP can be customized to implement a miniaturized ADCC assay for the evaluation of anti-CD38 efficacy. Microwells are selected to identify desired cell-cell interaction optimal patterns (e.g. featuring E/T co-localization patterns defined by the number of E/T cells or by the distance among E and T cells), and control conditions (e.g. microwells featuring only T cell types to evaluate direct drug cytotoxicity or microwells with E/T co-localization and no drug to measure spontaneous NK activity).

VALIDATION

Figure 1. Statistical model validation



R1 = ratio of effector cells in input cell population; R2 = ratio of target cells in input cell population; E:T = effector to target ratio; c = concentration of co-culture.

The model was validated in 3 cases:

i. E and T pure populations (R1=100%, R2=100%) (n=2)
ii. E cells coming from patient sample (WBC), T pure population (R2=100%) (n=2)
iii. E and T both coming from the original composition of a patient's sample (n=4).

Cases i) and ii) let the user the freedom to set the E:T ratio and the overall concentration (c), whereas in case iii) only the tuning of c parameter is allowed. The comparison between the predicted number of microwells featuring E/T co-localization ($E/T_{CF} > 0$) and the actual number obtained in the experiments gave a correlation coefficient $0.81 \leq R^2 \leq 0.98$ (n=8).

Table 1

Immune composition of tested patients		
Exp. ID	NK %	Plasma cells %
Exp. 1	19.8	9.7
Exp. 2	25.9	9.5
Exp. 3	18.2	6.8
Exp. 4	12.5	5.2
Exp. 5	18.1	6.2
Exp. 6	14.7	2.4
Exp. 7	10.1	1.8
Exp. 8	9.8	16.0

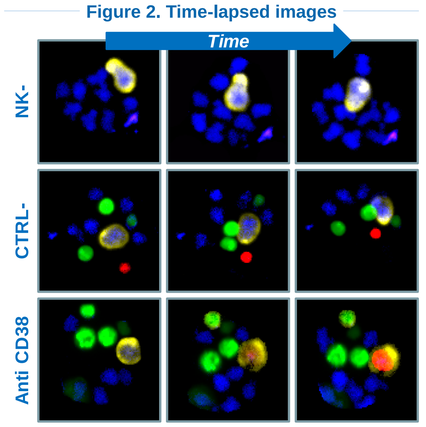
Immune composition of 8 MM patients' sample (Table 1). E/T cell co-localization occurs in at least 1% of microwells for all the 8 samples, making ICNP applicable with good statistical significance in the OMW system on ex-vivo clinical samples without any pre-enrichment.

ICNP IMAGE ANALYSIS

Figure 2. Time-lapsed images

Example of time-lapsed images analyzed with single-cell resolution and used for ICNP analysis. Each row in the image corresponds to a different condition: effect of Anti CD38 on a target cell belonging to a cluster with no effector cell (NK-), effect of the interaction between target and effector cells with no Anti CD38 (CTRL-), effect of Anti CD38 on a target cell interacting with effector cells (Anti CD38). Plasma cell death is visible in red.

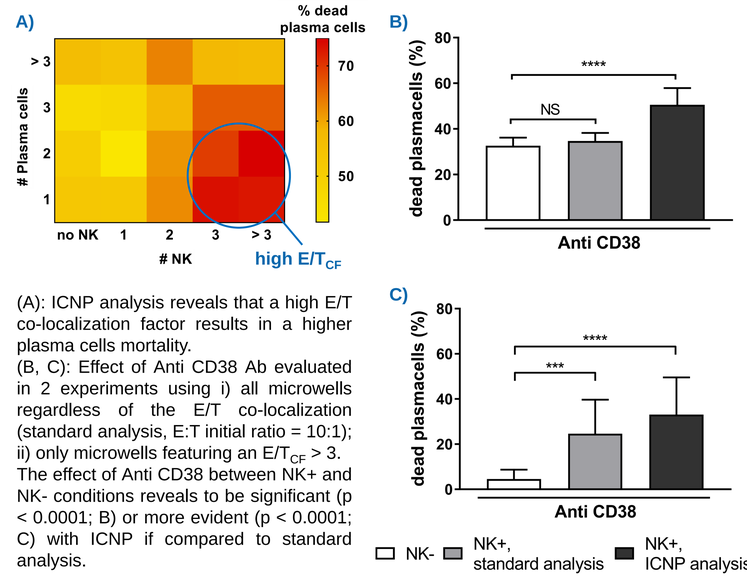
Time-lapse points: T=0h, T=1h, T=4h.



Green: NK **Yellow**: plasma cells **Blue**: other **Red**: dead cells

RESULTS

Figure 3. ICNP - Co-culture of U266 MM cell line with WBC

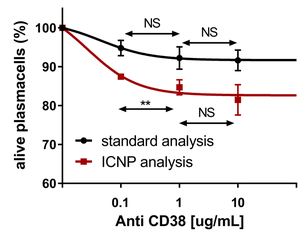


(A): ICNP analysis reveals that a high E/T co-localization factor results in a higher plasma cells mortality.

(B, C): Effect of Anti CD38 Ab evaluated in 2 experiments using i) all microwells regardless of the E/T co-localization (standard analysis, E:T initial ratio = 10:1); ii) only microwells featuring an $E/T_{CF} > 3$.

The effect of Anti CD38 between NK+ and NK- conditions reveals to be significant ($p < 0.0001$; B) or more evident ($p < 0.0001$; C) with ICNP if compared to standard analysis.

Figure 4. ICNP – Dose/Response experiment

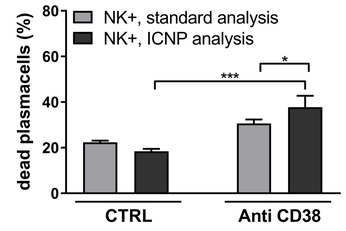


Mix of patient's WBC and U-266 MM cell line exposed to 3 different dosages of Anti CD38.

Initial E:T ratio was set to 8:1, according to our model, to maximize the number of microwells with $E/T_{CF} > 3$. ICNP analysis here considers patterns with E and T cells in contact and reveals a significant difference between 1ug/mL and 0.1 ug/mL dosages, that would be non-significant according to standard analysis.

Figure 5. ICNP – Complete MM patient sample

ICNP analysis in selected microwells featuring $E/T_{CF} > 3$ (4.97% of the total number of microwells) highlights a more pronounced ADCC effect with respect to a standard analysis ($ADCC_{standard\ analysis}$ = 10.64%, $ADCC_{ICNP}$ = 23.68%; where ADCC is the AntiCD38 cytotoxicity normalized to CTRL). Data shown refer to Exp 3, Table 1.



CONCLUSION

ICNP proved to enable a comprehensive profiling of the immune system by evaluating in one test the immune composition and the fitness of immune cells, both native and drug-treated. These results open the opportunity to develop functional precision medicine approaches for predicting patient's response to drugs with immune-mediated mechanisms of action.

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Disclosure: A. Bettelli, R. Ruggiano, S. Bocchi, L. Rocchi, A. Faenza, M. Bettelli, L. Rambelli, V. Guadagnuolo, N. Pecorari, L. Giulianelli, M. Pisani, 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.; E. Zamagni reports honoraria, advisory board and speaker bureau for Janssen, Amgen, Celgene, Sanofi and BMS and honoraria and speakers bureau for Takeda; M. Cavo reports consultancy, honoraria, membership on an entity's board of directors or advisory committees and speakers bureau for Janssen, Amgen, Celgene and Abbvie and honoraria, membership on an entity's Board of directors or advisory committees and speakers bureau for BMS, Sanofi, Novartis and Takeda; E. Borsi, C. Terragna and M. Martello have nothing to disclose.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 823614