Pre-therapeutic evaluation of patient-specific responses to immune-checkpoint inhibition in colorectal cancer

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Background

Cancer immunotherapy has revolutionized treatment options for patients suffering from immunogenic tumors, among them melanoma, bladder and lung cancer. In the context of colorectal cancer (CRC), the administration of checkpoint inhibitors (CPIs) was shown to induce durable clinical responses in patients with mismatch repair deficient and microsatellite instable tumors, a though very small fraction of all CRC subtypes. To ameliorate response rates in metastatic CRC, various therapeutic strategies are currently being investigated to increase the immunogenicity of mismatch repair proficient (pMMR)/ microsatellite stable (MSS) CRC tumors and to set the ground for immunotherapy. Several conventional therapies approved for treating CRC patients are classified as immunogenic cell death inducers and may aid in priming cytotoxic T cells to the patient's tumor.

IndiTreat® - the functional "Matchmaker" for colorectal cancer

At 2cureX, we aim to pre-therapeutically measure the potential responsiveness of a CRC patient to various drug therapy options to support the oncologists in identifying the best suitable treatment regimen. Our functional IndiTreat® assay system allows for testing of chemotherapeutic agents, targeted therapies and combinations thereof against 3D-microtumors (tumoroids), which we derive from CRC tissue or liver metastases (Figure 1).

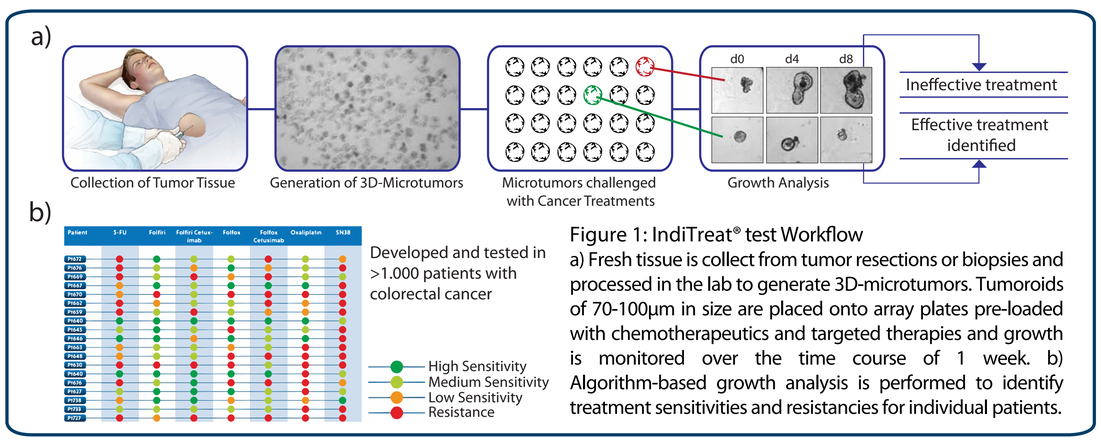

Figure 1: IndiTreat® test Workflow
a) Fresh tissue is collect from tumor resections or biopsies and processed in the lab to generate 3D-microtumors. Tumoroids of 70-100µm in size are placed onto array plates pre-loaded with chemotherapeutics and targeted therapies and growth is monitored over the time course of 1 week. b) Algorithm-based growth analysis is performed to identify treatment sensitivities and resistancies for individual patients.

To broaden the applicability of the IndiTreat® test, the present study aims to adapt the assay system to functional testing of checkpoint inhibitors in the context of pMMR/MSS CRC, subsequent to standard of care therapy.

Material & Methods

To assess the potential efficacy of immune oncology (IO) interventions for individual patients, we co-culture tumoroids and (in vitro expanded) autologous peripheral blood mononuclear cells (PBMCs) and monitor immune-mediated killing of tumoroids after exposure to checkpoint inhibitors.

Sample collection and in vitro culture of autologous immune effector cells

Over the period of 1 year, tissue samples were collected from 24 patients diagnosed with pMMR/MSS colorectal adenocarcinoma, some of which having undergone neo-adjuvant chemotherapy. While proliferating tumoroid cultures could be established from almost 60% of resected tumor samples, 3D-microtumors and matching blood samples were only obtained in 6 cases, i. e. 25% of patients (Table 1). Tumoroids were grown, maintained and propagated as previously described [1,2]. Once expanded to sufficient numbers, a fraction of tumoroids was treated with IFNγ for 24 hours to evaluate surface expression levels of HLA class I molecules and PD-L1, a known ligand of the immune checkpoint receptor PD-1 on T cells (Figure 2). Co-cultures of tumoroids and autologous PBMCs were performed pursuing two parallel strategies (Figure 3): We co-cultured tumoroids and ex vivo PBMCs for 24 hours followed by analysis of T cells ("week 0" co-culture). Next to that, a fraction of ex vivo PBMCs was cultivated in the presence of fragmented tumoroids and Pembrolizumab according to the protocol of Dijkstra and colleagues [3], prior to performing 24 - 72 hours co-cultures of tumoroids and in vitro expanded PBMCs ("week 2" co-culture). Data analysis of co-cultures was performed by flow cytometry and (fluorescence) imaging.

Patient #	Date	Pre-treated?	Blood available ?	HLA evaluated?	Co-culture performed?	Remarks
1	28./29.11.18	yes	yes	yes	autologous	
2	02/03.12.18	no	yes	yes	autologous + allogeneic	
3	02/03.01.19	no	yes	yes	autologous	
4	18./22.01.19	no	yes	no		tumoroids lost in the process of maintenance
5	29./30.01.19	no	yes	no		bacterial contamination
6	05./06.02.19	no	yes	yes	autologous	
7	19./20.02.19	no	yes	no		tumoroids lost in the process of maintenance
8	21.02.19	no	no	no		
9	25./26.02.19	no	yes	no		tumoroids lost in the process of maintenance
10	12./13.03.19	no	yes	no		tumoroids lost in the process of maintenance
11	04.04.19	no	no	no		
12	15.04.19	no	no	no		
13	16.04.19	no	yes	no		tumoroids lost in the process of maintenance
14	02./03.05.19	yes	yes	yes	autologous	
15	23./24.05.19	no	yes	no		tumoroids lost in the process of maintenance
16	26./27.05.19	no	no	no		
17	27./28.05.19	no	no	yes		
18	29.06.19	yes	yes	no		bacterial contamination
19	02.08.19	yes	no	no		
20	14.08.19	no	yes	no		tumoroids lost in the process of maintenance
21	23.08.19	?	no	no		
22	18.10.19	no	no	no		
23	22.10.19	no	yes	no		
24	29.11.19	no	no	no		tumoroids not formed

Table 1: Peripheral blood and samples of resected CRC tumor tissue where collected according to the vote of the ethical committee in Hamburg and after obtaining informed patient consent. Blood was drawn prior to surgery, processed within a few hours and cryopreserved until the start of co-cultures. We obtained peripheral blood in 15 cases, tumoroids formed and grew to sufficient numbers in 14 cases and both PBMCs and tumoroids were available for experimental purposes in 6 cases. Autologous co-cultures of tumoroids and PBMCs have thus far been set up for 5 patients.

[1]Jeppesen et al. (2017) Short-term spheroid culture of primary colorectal cancer cells as an in vitro model for personalizing cancer medicine. PLOS One
[2]Árnadóttir et al. (2018) Characterization of genetic intratumor heterogeneity in colorectal cancer and matching patient-derived spheroid cultures. Molecular Oncology
[3]Dijkstra et al. (2018) Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. Cell

Results

Expression of HLA class I molecules and ligands of immune checkpoint molecules PD-1 and TIGIT on tumoroids were elevated under pro-inflammatory conditions (Figure 2).

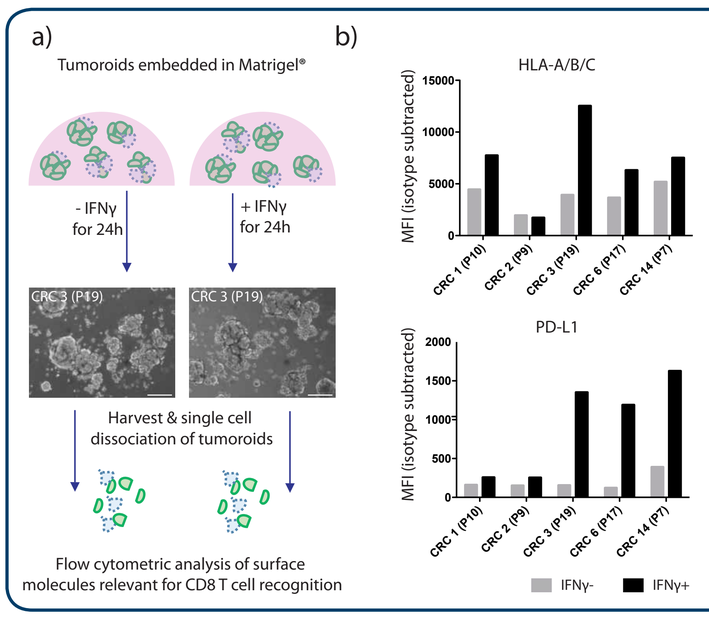

Figure 2: Flow cytometric analysis of single cell dissociated tumoroids
a) Tumoroids embedded and grown in Matrigel® are cultured in the presence or absence of 200ng/ml IFNγ prior to photodocumentation (shown here: tumoroids of patient CRC 3 in passage (P) 19; scale bar = 200µm), recovery from Matrigel®, trypsinization and flow cytometric analysis. b) Expression of HLA class I molecules was detected on all analyzed samples to varying degrees and could, with exception of CRC 2, further be induced by IFNγ. Baseline expression of PD-L1 on single cell tumoroids was low but readily inducible by IFNγ on CRC 3, CRC 6 and CRC 14. Occasionaly , surface expression levels of TIGIT ligands PVR and PVR-L2 were also evaluated (data not shown).

In vitro expansion of tumor-specific T cells in the presence of tumor fragments and Pembrolizumab modulated protein expression levels of PD-1, TIGIT, Granzyme B and IFNγ in the subset of $CD8^+$ T cells, when evaluated subsequent to a 24h co-culture with autologous tumoroids. Pre-cultured PBMCS of patient CRC 14, having received 5 cycles of neoadjuvant FOLFOX, converged into clusters enclosing tumoroids by day 3 of the co-culture. Furthermore, production of both cytolytic Granzyme B and pro-inflammatory IFNγ was detected in the great majority of $CD8^+$ immune effector cells of CRC 14 (Figure 3).

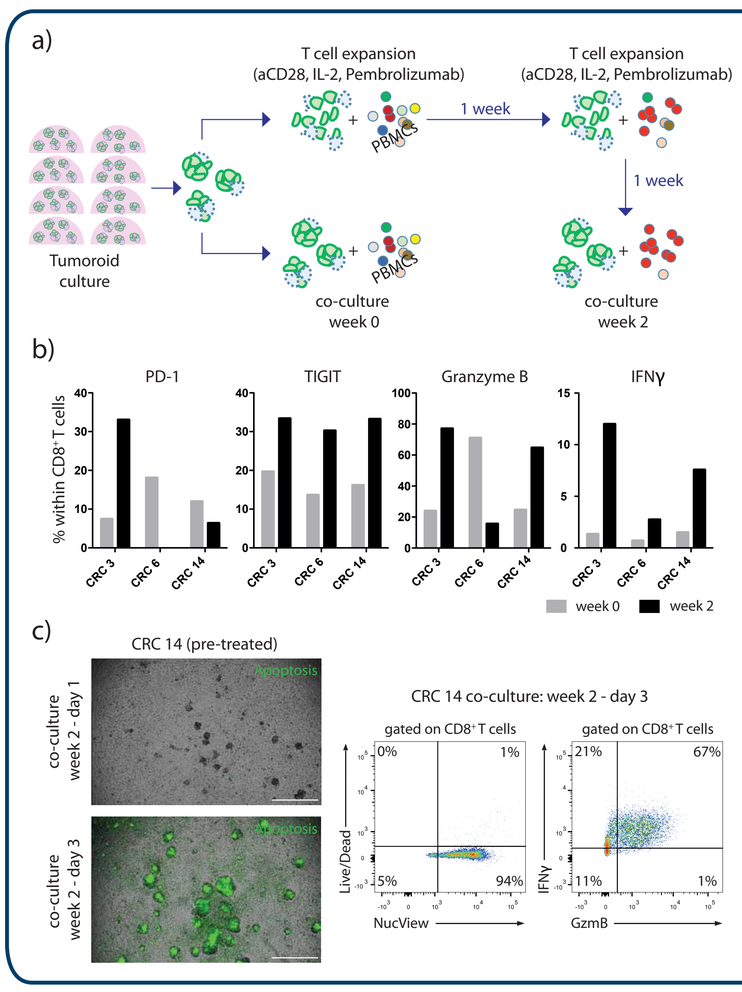

Figure 3: Autologous co-culture of tumoroids and PBMCs
a) 3D-microtumors are recovered from Matrigel® and filtered (70-100µm fraction) prior to co-culture with PBMCs (week 0). Alternatively, tumoroids are fragmented to support expansion of tumor-specific T cells over the course of a 2 week culture on aCD28-coated plates in the presence of 150U/ml IL-2 and 20µg/ml Pembrolizumab (aPD-1). Expanded PBMCs are harvested and once more co-cultured with 70-100µm tumoroids (week 2). Co-cultures are performed in 96-well plates, seeding 50 tumoroids and 100.000 PBMCs per well. b) Frequencies of $PD\text{-}1^+$, $TIGIT^+$, Granzyme B^+ and $IFN\gamma^+$ $CD8^+$ T cells were evaluated after 24 hours of co-culture. While expression levels of PD-1 and intracellular Granzyme B varied across samples and time points, TIGIT and intracellular IFNγ were more highly expressed by $CD8^+$ T cells subsequent to a 2 week pre-culture with fragmented tumoroids. c) 2 week expanded immune effector cells of CRC 14 were co-cultured with autologous tumoroids and apoptosis was visualized by detection of caspase-3/7 activity (NucView® 488) using a Cytation 1 imaging reader (scale bar = 1000µm). By day 3, PBMCs clustered in areas of tumoroids with clusters emitting strong fluorescence signals. At this time, 94% of viable $CD8^+$ T cells stained positive for NucView® and intracellular IFNγ was produced by 88% of viable $CD8^+$ cells.

Conclusion & Outlook

Tumoroids recapitulate the highly individual disease of cancer patients and constitute a valuable platform for evaluating different aspects of immune-mediated tumor cell recognition and killing. Additional experiments will be performed to elucidate potential differences regarding the feasibility of expanding tumor-specific T cells from peripheral blood of treatment naïve pMMR/MSS CRC patients, versus patients having undergone (immune stimulatory) neoadjuvant chemotherapy. Further, we aim to broaden our study by supplementing PBMC in vitro expansion cultures and/or tumoroid co-cultures with a number of different checkpoint inhibitors, including monoclonal antibodies targeting the TIGIT-PVR/PVRL2 axis. Improved visualization and quantification of T cell-tumoroid interactions is currently under investigation employing additional imaging systems such as the IncuCyteZoom platform.

Pre-therapeutic in vitro testing of patient-specific responses to CPIs may evantually allow for stratification of pMMR/MSS CRC patients according to their likelihood to benefit from IO therapy. This approach holds the potential to make these powerful drugs available to more patients suffering from CRC.

IndiTreat® Individualizing Cancer Treatment

2cureX has developed a test called IndiTreat® (Individual Treatment Design), which is a patented method for selecting the right drug for the right patient. IndiTreat® establishes thousands of 3D micro-tumors that are functionally similar to the patient's tumor. From a large panel of approved cancer treatments IndiTreat® selects the best treatment for the individual patient. IndiTreat® is expected to become a standard tool in the treatment design for cancer patients. IndiTreat® is currently being clinically investigated in colorectal cancer, ovarian cancer, pancreatic cancer and preventive medicine. The company is listed at the Nasdaq First North stock exchange in Stockholm (symbol "2CUREX")

* Authors have declared a financial interest in products/processes described in this poster

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