

BACKGROUND

Despite the increasing availability of therapeutics, lung cancer is one of the deadliest cancers in the world. Research focus on looking for better therapeutics, but less than 10% of the anticancer drugs that enter clinical trials ever reach the market. This strongly suggests that the current in vitro and preclinical models are not reliable predictors of the actual in vivo efficacy and toxicity of anticancer drugs in humans.

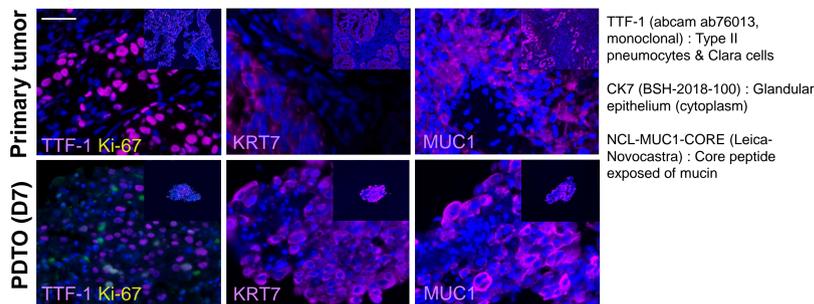
Patient-derived tumoral organoids (PDTO) offers the ability to maintain patients' heterogeneity and tumoral markers. Moreover, it is possible to recreate tumor microenvironment (TME) and external factors that actively participate to tumoral progression, especially immune cells. Immune cells comprise different populations that participate to promotion or regression of lung cancer¹.

For our study, we focus on of the emergent immunomodulatory treatment: the development of oncolytic virotherapy based on viral vectors. The oncolytic viruses propose to target specifically cancer cells by exerting oncolysis and modulating the tumor microenvironment to boost immune anti-tumoral responses². Although all these potentials, their therapeutic efficacy needs to be improved. Different factors affect their intra-tumoral spreading and consequently their efficacy: (i) the tumor itself (tumor heterogeneity, dense network of extracellular matrix ...) and the (ii) the immune tumor-microenvironment (infiltrating immune cells).

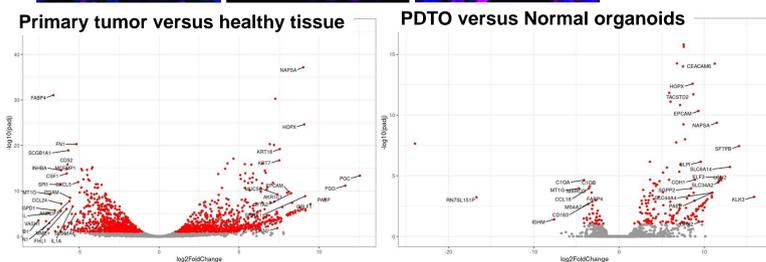
We will present our in vitro platform to assess oncolytic virotherapy, in a more relevant way, based on a human model.

1. THE PATIENT-DERIVED TUMORAL ORGANOID (PDTO) AT DAY 7

a. Tumoral markers are assessed by IF (primary tumor slide versus PDTO slide at day 7 of the culture) & 3' RNA sequencing

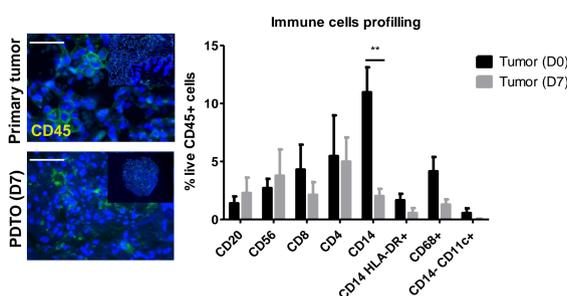


TTF-1 (abcam ab76013, monoclonal) : Type II pneumocytes & Clara cells
CK7 (BSH-2018-100) : Glandular epithelium (cytoplasm)
NCL-MUC1-CORE (Leica-Novocastra) : Core peptide exposed of mucin

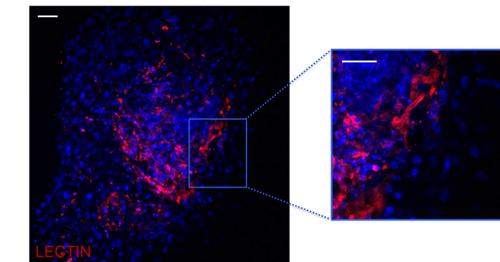


- ✓ Tumoral markers are retained in the PDTO (transcriptomic level)
- ✓ Tumor infiltrating immune cells are kept in culture over 7 days (CD4+, CD8+ cells...)
- ✓ A μ vasculature is formed by the addition of ad-MVs in PDTO (lectin marker shows the presence of a lumen within endothelial cells)

b. Tumor-infiltrating cells are verified by IF (CD45 marker) and by flow cytometry

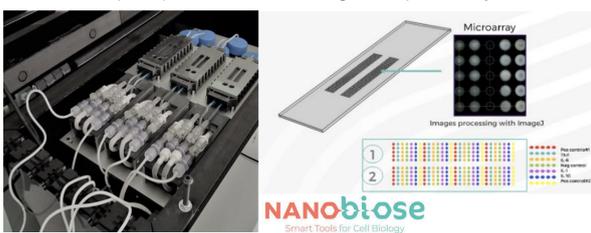


c. Functional endothelial cells (ad-MVs) were characterized by IF (lectin marker)

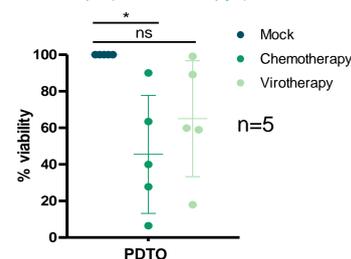


3. IN FLUIDIC CONDITIONS

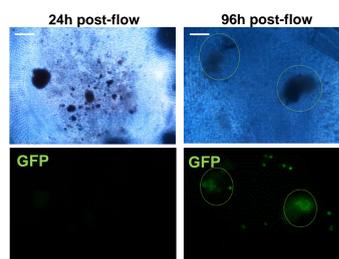
Flow rate : 600 μ L/h (established according to the pulmonary blood flow rate)



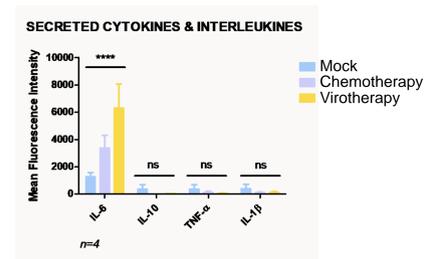
a. PDTO' sensitivity to chemotherapy (cisplatin 9 μ M-pemetrexed 24 μ M) & virotherapy (MOI = 1.10⁵ PFU)



b. Infection after 1h of virotherapy exposure : is there an infection ability in fluidic conditions ?



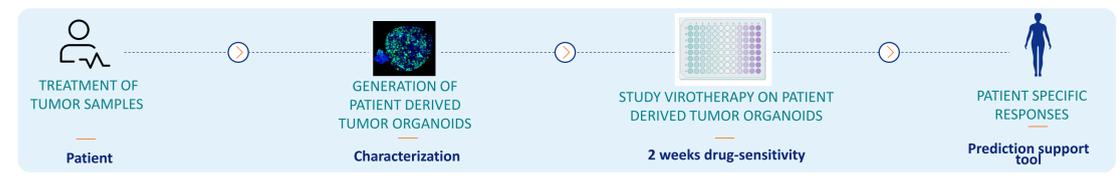
c. Direct monitoring of the secreted cytokines & interleukins



- ✓ Drug sensitivity in fluidic conditions (by mimicking 1h of IV injection)
- ✓ PDTO are more sensitive to chemotherapy
- ✓ Chemotherapy and virotherapy increase the levels of secreted IL-6 compared to IL-10, TNF- α and IL-1 β

OBJECTIVES

1. Formation and Characterization of the PDTO model
2. Assess PDTO's permissivity to oncolytic virotherapy
3. Assess PDTO's response in fluidic conditions (to mimick intravenous (IV) administration)



METHODS AND TIMELINE

Human specimen & generation of PDTOs

PDTOs were formed from patients' tumor samples with lung adenocarcinoma (planned for the 1st line surgery). Patients' informed consents were managed by the CRB (Centre de Ressources Biologiques des Hôpitaux Universitaires de Strasbourg). Human samples were digested. The final suspension was then co-cultured with adipocytes-derived microvessels (ad-MVs) ULA plates (96 wells). PDTOs were formed after 7 days of incubation at 37° C.

Preparation of treatments with oncolytic viruses (cisplatin – pemetrexed)

Treatments were diluted in cell culture media according to the titer and concentrations to be tested.

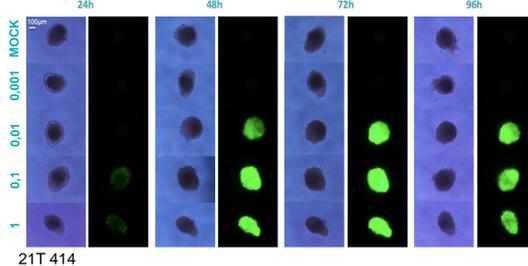
Day 0
Dissociation of tumor samples & Generation of PDTO

Day 7
Treatment with oncolytic viruses (Multiplicity of infection (MOI) = 0,1)
Incubation during 96h 37° C for static conditions (objective 2)
Incubation during 1h at 37° C for fluidic conditions (objective 3)

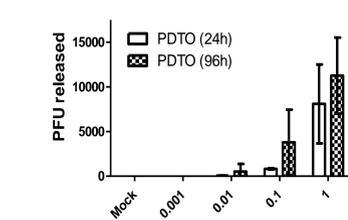
Day 11
End of treatment
○ Infection ability
○ Replication
○ Cytotoxic activity
○ Immune cells activation ?

2. ASSESS PDTO' S PERMISSIVITY TO ONCOLYTIC VIROTHErapy (N=3)

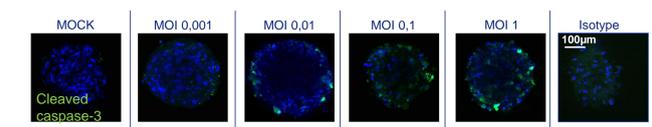
a. Infection ability : GFP expression



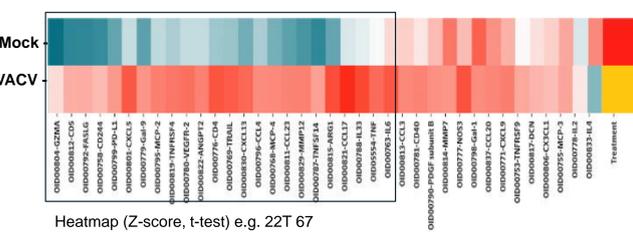
b. Replication ability ? (plaque assay, n = 3)



c. Cytotoxic activity : Caspase 3 activity by IF, 96h post-infection



d. Immune cells activation (Protein profiling using Olink Technology)



- ✓ PDTO are permissive to oncolytic viruses (at MOI 0,01 to 1, from 72h post-infection)
- ✓ In human tumoral cells : amplification of the virus particles is observed (96h)
- ✓ Expression of cleaved caspase-3 is increased according to the MOI
- ✓ After 96h of infection, oncolytic virotherapy can enhance proteins such as Granzyme A, CD5, CD244, CD4 and chemokines (CXCL5, CCL17, TNF ...) implied in immune cells response activation

CONCLUSION & PERSPECTIVES

- ✓ Our PDTO model faithfully recapitulate patients' primary sample features (tumoral markers at the IF and transcriptomic level and tumor-infiltrating immune cells)
- ✓ Ad-MVs can provide a microvasculature within PDTO, in the aim to perfuse the 3D organoid
- ✓ PDTO are permissive to virotherapy
- ✓ The virotherapy can awaken apoptotic pathway to exert cytotoxic activity (cleaved caspase-3) and upregulate some cytokines & interleukins implied in immune cells activation, at MOI = 0,1
- ✓ Mimicking IV injection (1h) of virotherapy, the oncolytic virus could infect PDTO.
- ➔ Specific patient responses can be deciphered in this in vitro platform
- x Physiological barriers affecting virotherapy are missing in this in vitro platform, including neutralizing antibodies, blood cells elements, circulating immune cells among other parameters.

