3D organoids derived from patients' lung tumours: a tool for investigating the potential of oncolytic viruses

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INTRODUCTION AND RATIONALE

Lung cancer remains a major cause of cancer death, non-small cell lung carcinoma (NSCLC) with a survival rate below 20% [1]. Lung cancer has been shown to be immunogenic and responsive to immunotherapy. Thus, immune checkpoint inhibitors significantly improved survival rate, reaching up to 40% OS in patients with high levels of tumor PD-L1 expression. However, most of NSCLC patients still fail to respond due to the development of primary or secondary resistance [2]. In this context, alternative therapies are needed. Oncolytic viruses might represent a promising option by their ability to replicate specifically in, and to kill tumoral cells, and their ability to largely engage the immune system. T-VEC has been approved in the USA and in Europe for intra-tumoral treatment of melanoma [3]. The progress of oncolytic virotherapy in the field of NSCLC will require more innovation. In this perspective, a 3D tumor model has been established from patients' lung biopsies to recapitulate the complex architecture and microenvironment of the lung cancer, and better understand the way OVs penetrates and spread into the tumor, and the way they interact with immune mechanisms in situ. The potential of armed Vaccinia vectors developed by Transgene (invirIO™ platform) will be studied either as a monotherapy, and in combination or as adjuvant therapy with other cytotoxic therapies or sequentially after induction of chemoresistance phenotype in order to override drug resistance and enrich the therapeutic response [4].

MATERIAL & METHODS

Human specimens

Patients' derived organoids (PDO) were formed from either lung's cancer biopsies, from patients planned for 1st line surgery. Patients' informed consents were managed by the CRBS (Centre de Ressources Biologiques des Hôpitaux Universitaires de Strasbourg).

Tissue preparation and culture of PDOs

Human samples were washed in PBS and enzymatic digestion was performed at 37° C for 1h with intermittent agitation. After incubation, the reaction media containing samples was transferred to a Falcon tube through 70µM cell strainers. The strained cells were centrifuged and the pellet was resuspended in 1mL of DMEM-F12 (Lonza). Other cell types such as cancer-associated fibroblasts, human pulmonary alveolar cells type II and human vein umbilical endothelial cells were added to reconstitute the microenvironment. The cell suspension was then seeded for a final volume of 200µL in Thermoscientific™ Nunclon™ Sphera™ plates (96 wells). PDOs were formed after 5 days of incubation at 37° C.

Histology and imaging

Tumoral PDOs were fixed in 4% paraformaldehyde (PFA). They were dehydrated before paraffin inclusion in order to further perform immunohistochemistry (IHC). For IHC staining, the samples were acquired in the BOND RxM automate. Slides were saturated with H_2O_2 solution during 10min followed by a 10min incubation with goat serum (Sigma®, G6767). Then following primary antibodies were incubated during 45min : anti-TTF1 (1:250 ; Abcam® ab76013), anti-PD-L1 (1:200 ; Cell Signaling®, 13684). Primary antibodies were revealed with tertiary antibody Novolink (Leica®, RE7161). Signal is revealed by TSA-Cy3 or Cy5 (Perkin Elmer®, SAT704A). Nucleis were stained with Hoechst (1:10 000 ; Thermo Scientific® 62249) during 10min. After a drug therapy treatment, the samples were incubated with the primary antibody anti-caspase 3 clived (1:1000 ; Cell Signalling® 9661) and anti-gfp (1:250; Cell Signalling® 46890).

Drug screening

Once tumoral PDOs were formed, treated either with chemotherapy alone or in combination with OV (a recombinant Vaccinia virus Copenhagen strain deleted in Thymidine Kinase and Ribonucleotide Reductase that expresses GFP). Duration of treatment is 96 hours. After 96h days of treatment, the medium is replaced by fresh media containing 25% of CellTiter-Blue® reagent to measure cell viability. The plates are agitated for 1min at room temperature prior to incubation at 37° overnight. Then, next morning, the fluorescence reading is proceeded.

Virus quantification

To assess virus proliferation, 24h and 96h post-infection, PDO and supernatant were harvested and frozen at -80° C. Virus progeny was quantified by plaque assay on Vero cells, on 6 wells plates.

Secretome analysis

Concentrations of soluble factors in the supernatants were detected by the multiplexed Luminex MAGPIX (according to the manufacturer's instructions).

RESULTS - DISCUSSION

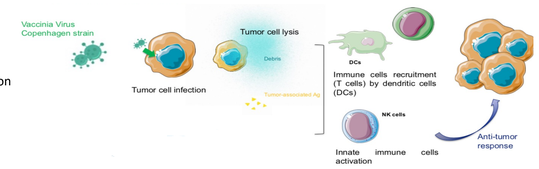
BACKGROUND

Oncolytic Vaccinia Virus features

- Recombinant Vaccinia virus Copenhagen strain
- deleted in Thymidine Kinase and Ribonucleotide Reductase genes (VVTK-RR-)

⇒ inhibits viral replication in normal cells while retaining its therapeutic replication in tumor cells.

- Vaccinia virus presents a large double-stranded DNA genome (~ 190 kb)
- Immunostimulatory effects (as illustrated besides)



MONOTHERAPY

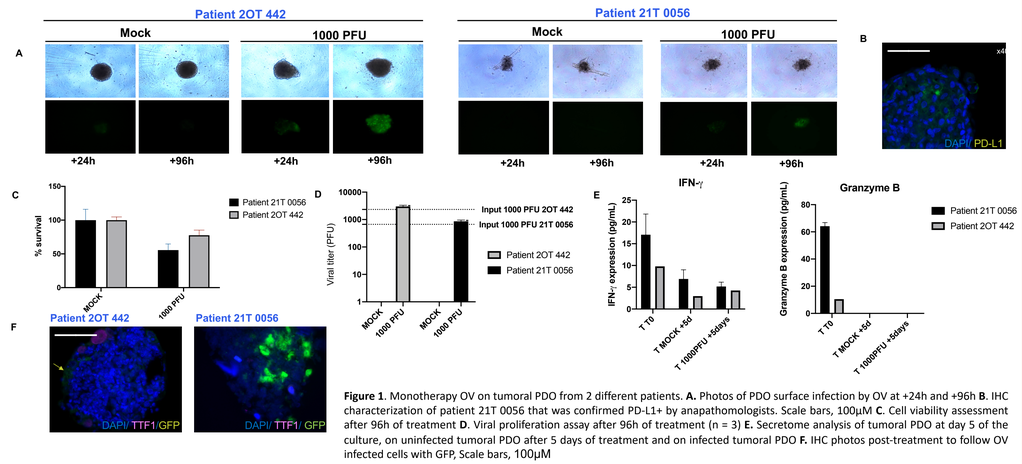


Figure 1. Monotherapy OV on tumoral PDO from 2 different patients. **A.** Photos of PDO surface infection by OV at +24h and +96h **B**. IHC characterization of patient 21T 0056 that was confirmed PD-L1+ by anapathomologists. Scale bars, 100µM **C**. Cell viability assessment after 96h of treatment **D**. Viral proliferation assay after 96h of treatment (n = 3) **E.** Secretome analysis of tumoral PDO at day 5 of the culture, on uninfected tumoral PDO after 5 days of treatment and on infected tumoral PDO **F.** IHC photos post-treatment to follow OV infected cells with GFP, Scale bars, 100µM

OV in monotherapy decreases the cell viability of tumoral PDO

COMBINATION

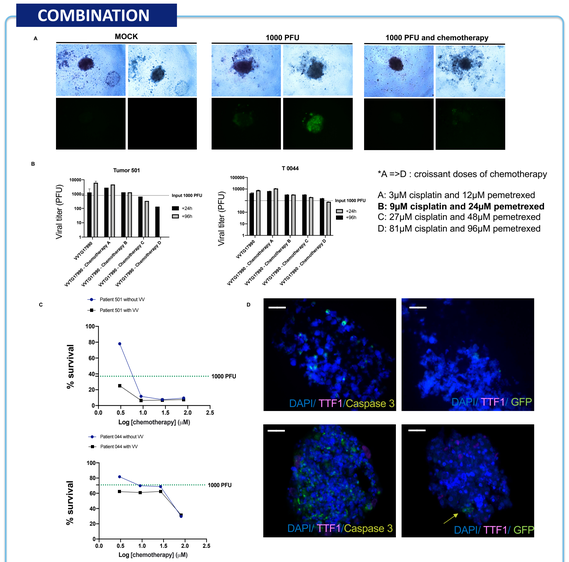


Figure 2. Combination strategy on tumoral PDO from 2 different patients. **A.** Photos of PDO surface infection by OV at +24h and +96h **B**. Viral proliferation assay after 96h of treatment (n=3) **C**. Cell viability assessment after 96h of treatment (dotted line represents viability with OV only) **D**. IHC photos post-treatment to stain apoptosis with caspase 3 and OV infected cells with GFP, Scale bars, 100µM

- Low doses of chemotherapy in monotherapy are less efficient than OV
- Combination with OV : no additional effect observed
- Drugs responses are different on the 2 patients : patient 501 is more sensitive to patient 044

The first generation of chemotherapeutics in NSCLC (including cisplatin) showed low response rates ranging from 15 to 25% when used as monotherapy [6]. Many lung cancer treatment protocols recommend a combined chemotherapy plan with two platinum-based anticancer drugs (e.g., Paclitaxel, docetaxel, gemcitabine or pemetrexed) to improve the response rates up to 35%, yet, It is not sufficient. For strategy combination, we aim to study if an additional effect is observed when OV is combined to chemotherapy, the first-line treatment for cancer patients. Here, we show on 2 adenocarcinoma patients that combination with chemotherapy (cisplatin and pemetrexed) decreased tumor cells viability at high doses. At high doses of chemotherapy, OV didn't add value to chemotherapy effect. Indeed, we observe that chemotherapy exerts a cytotoxic effect as the dose is higher (caspase staining is more important). Besides, we observe that OV infected only peripheral cells.

DEMOGRAPHICS

4 patients were used for these experiments.

	20T 442	21T 0056	21T 0501	21T 0044
Gender	Male	Male	Male	Male
Age (years)	63	77	74	77
Histological subtype	Adenocarcinoma	Epidermoid carcinoma	Adenocarcinoma solid type	Acinar adenocarcinoma
Pre-operatory treatments	Neo-adjuvant chemotherapy (cisplatin + vinorelbine)	Ipilimumab & cemiplimab combined with carboplatin and gemcitabine	No treatment (indication RCP : Adjuvant chemotherapy)	No treatment

CONCLUSION

Treatment with chemotherapy or OV alone demonstrated an ability to control tumor growth, the combination of cytotoxic drugs and OV failed to demonstrate additional benefit. The reason might be that chemotherapy negatively impacted the replication of OV in the tumor burden. The challenge will be to find the balance between cytotoxicity and OV infection. Croissant doses of OV need to be evaluated in combination with chemotherapy to observe a potential additional effect. Another interesting strategy besides combination strategy is to study OV potential therapeutic effect on resistant patients. Most of the patients under chemotherapy develops acquired resistance, this highlights the need for using potential therapeutics such as OV to bypass the resistance.

Perspective: Supplementary adenocarcinoma patients are being assessed and will complete the results to add more robustness to these observations.

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DISCLOSURES

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