

TROCEPT - A tumor-targeted precision immunovirotherapy enabling $\alpha\text{v}\beta 6$ integrin-positive tumor-localized expression of an immune checkpoint inhibitor following intravenous delivery

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1. TROCEPT addresses major limitations of other viral technologies

Oncolytic viruses for the treatment of cancer promise much but have so far failed to show significant efficacy. TROCEPT overcomes the main limitations of other systemically delivered viruses ^{1,2,3}.

TROCEPT is an adenovirus type 5 (Ad5) engineered:

- 1) To no longer infect normal human tissues such as the liver, through native tropisms
- 2) To only enter cells expressing a tumor marker, $\alpha\text{v}\beta 6$ integrin
- 3) To only replicate within tumor cells
- 4) To deliver therapeutic transgenes

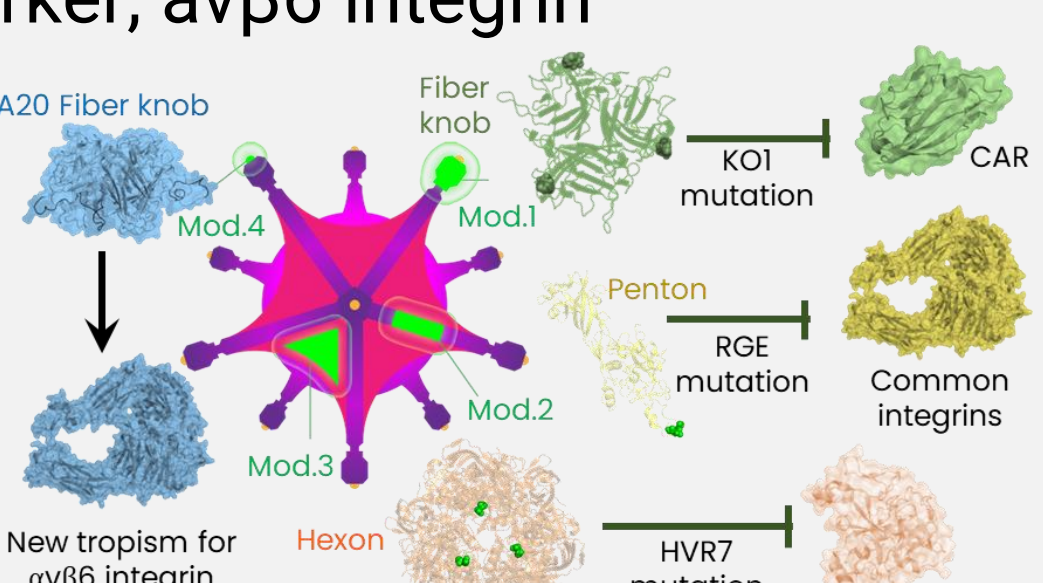


Figure 1: TROCEPT has hexon, penton and fibre knob native receptor binding ablations to remove all known natural tropisms and stop infection of normal tissues. TROCEPT has an introduced $\alpha\text{v}\beta 6$ integrin specificity to target the majority of solid tumors^{1,2,3}

2. TROCEPT is specific for the cancer marker, $\alpha\text{v}\beta 6$ integrin

TROCEPT infects tumor cell lines expressing $\alpha\text{v}\beta 6$ integrin in an expression-dependent manner.

Figure 2 demonstrates that TROCEPT infects, (as measured by qPCR for hexon) and elicits transgene payload production in (as measured by GFP expression) $\alpha\text{v}\beta 6$ integrin-expressing cells and does not infect $\alpha\text{v}\beta 6$ integrin-negative cells. TROCEPT does not infect cells through the native adenovirus receptors CAR or common integrins, or via FX.

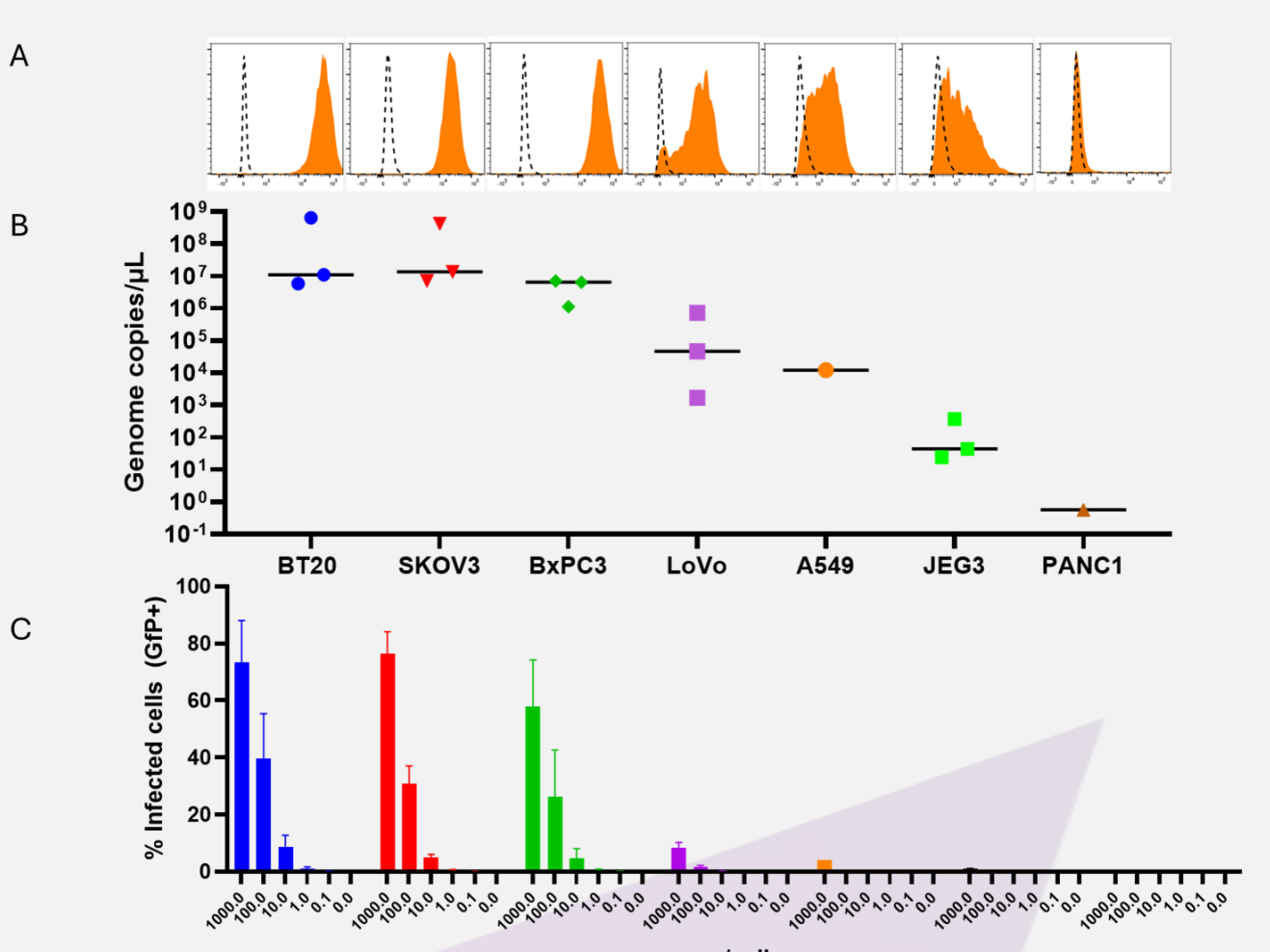


Figure 2: A) $\alpha\text{v}\beta 6$ integrin expression by flow cytometry on cell lines. All these cell lines express CAR and $\alpha\text{v}\beta 5$ (data not shown). These cell lines were incubated with TROCEPT-GFP at 1000, 100, 10, 1 or 0.1 virus particles (vp) per cell for 3 hours, cultured for 48 hours and assessed for infection by B) qPCR for viral hexon at 10vp and C) flow cytometry for transgene payload GFP expression. n=3 except A549 and PANC1 n=1.

3. $\alpha\text{v}\beta 6$ integrin is highly expressed on epithelial cancers

$\alpha\text{v}\beta 6$ integrin is highly expressed in a range of epithelial tumors, confirmed by in-house immunohistochemistry of tumor samples (Table 1 and Figure 3). A selection of these indications will be evaluated as part of a phase I clinical study.

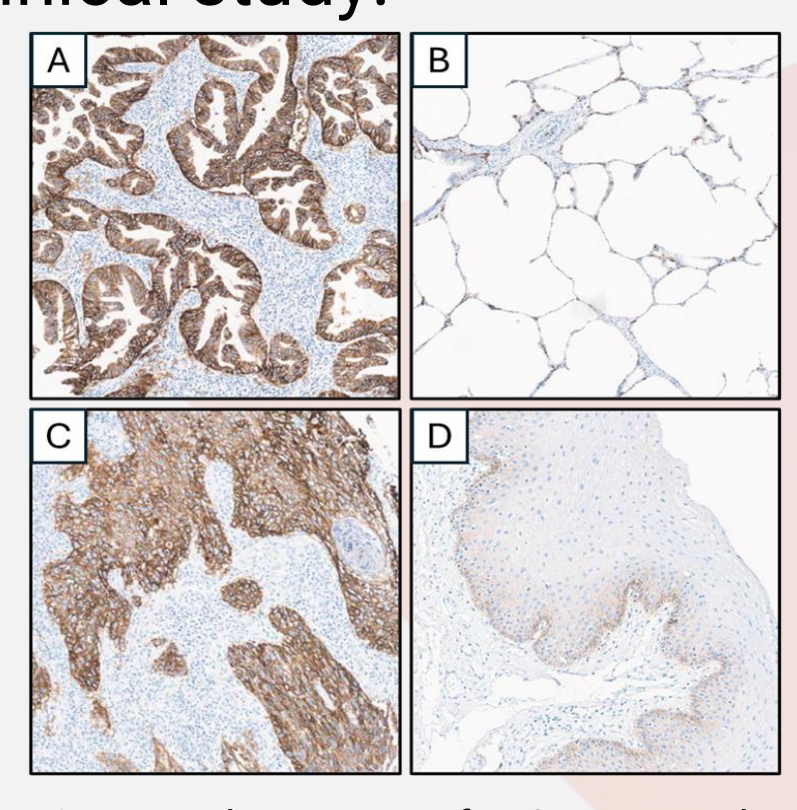


Figure 3: Example staining of $\alpha\text{v}\beta 6$ integrin by immunohistochemistry in A) lung adenocarcinoma, B) normal lung, C) esophageal squamous cell carcinoma and D) normal esophagus

Tumor type	N	% positive by IHC
Head & Neck	96	88
Lung Adenocarcinoma	50	96
Lung Squamous Cell Carcinoma	34	79
Bladder Carcinoma	58	95
Pancreatic Ductal Adenocarcinoma	35	97
Breast Invasive Carcinoma	36	72
Colon Adenocarcinoma	37	65
Cholangiocarcinoma	59	90
Endometrial Adenocarcinoma	38	79
Stomach Adenocarcinoma	34	62
Ovarian Adenocarcinoma	37	46

Table 1: Frequency of $\alpha\text{v}\beta 6$ integrin positive tumors within n samples of the stated tumour types by immunohistochemistry

4. TROCEPT kills tumor cells and NOT normal cells *in vitro*

TROCEPT replicates in tumor cells resulting in lysis, however no cell death is observed in normal cells from a range of cell types and organ systems. This is shown in Figure 4 by MTS assay and representative images demonstrating maintenance of the intact monolayer with the normal cells but not tumor cells.

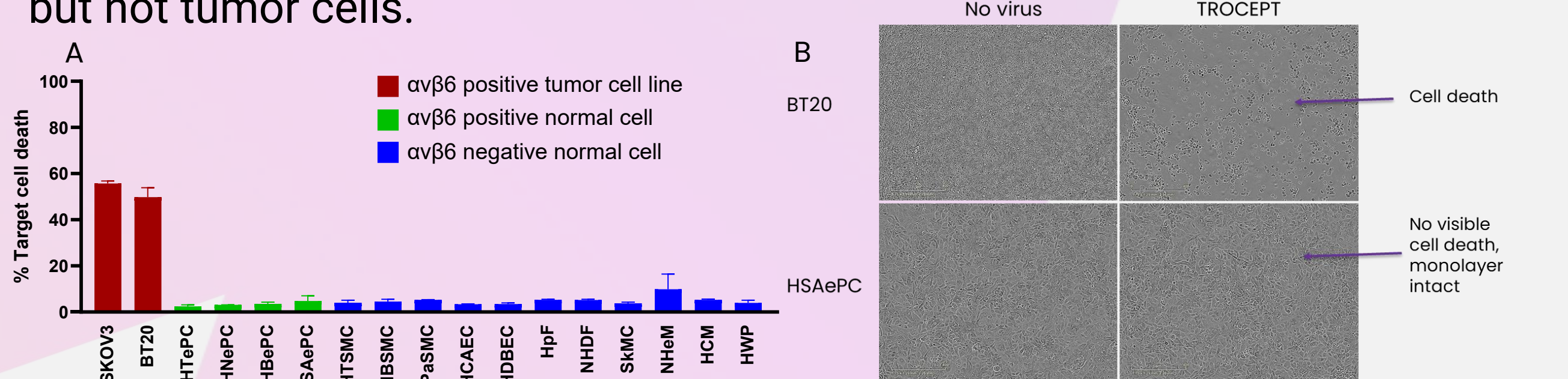


Figure 4: A) MTS assay demonstrating lysis of $\alpha\text{v}\beta 6$ integrin positive tumor cells but not $\alpha\text{v}\beta 6$ integrin-positive normal epithelial cells or $\alpha\text{v}\beta 6$ integrin-negative normal cells from a range of cell types at 5 days post infection by TROCEPT at 1 viral particle per cell. B) Phase contrast images of cell culture monolayers at 5 days post infection by TROCEPT showing loss of the BT20 breast carcinoma cell monolayer yet maintenance of the HSAePC normal small airway epithelial cell monolayer. Representative data of 3 independent experiments.

5. Single intravenous dose of TROCEPT controls tumors in multiple indications

Intravenous administration of GFP-expressing TROCEPT resulted in rapid oncolysis of large established $\alpha\text{v}\beta 6$ integrin expressing bladder, colorectal, ovarian, pancreatic and lung carcinomas (Figure 5A) and survival of mice (Figure 5B). No off-tumor effects were observed. Mice treated with vehicle demonstrated rapid tumor growth with 100% mortality.

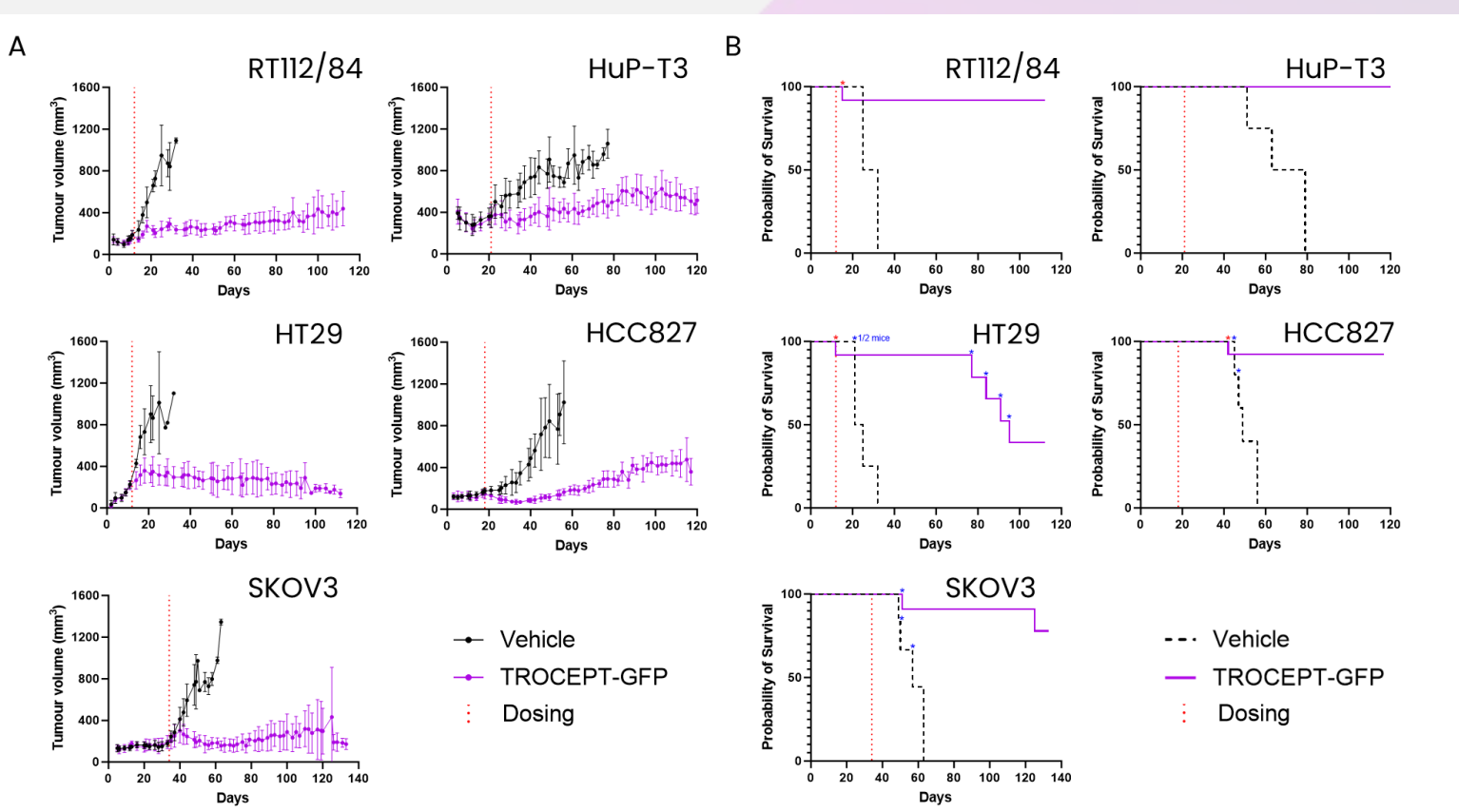


Figure 5: A) Tumor volume and B) survival plots of NSG mice with subcutaneous RT112/84 bladder, HT29 colorectal, SKOV3 ovarian, HuP-T3 pancreatic or HCC827 lung human tumor cell line xenografts were administered intravenously with a single dose of 1×10^{11} vp TROCEPT-GFP or vehicle control at the stated timepoints (when tumors reached approximately 200mm³). Unless stated otherwise mice were sacrificed when maximum tumor volume was reached. * sacrificed due to clinical observations, * sacrificed due to tumor ulceration.

6. TROCEPT enables tumor-specific transgene payload production *in vivo*

Administration of TROCEPT leads to tumor-specific transgene payload (luciferase) production which increases with time as TROCEPT replicates in the tumor only, compared to unmodified Ad5 which leads to body-wide transgene payload production (Figure 6). Both viruses infect and replicate in the tumor, building signal over time, however luciferase activity is observed only in the tumor only with TROCEPT. This also confirms the removal of the native tropisms in TROCEPT and specificity for $\alpha\text{v}\beta 6$ integrin.

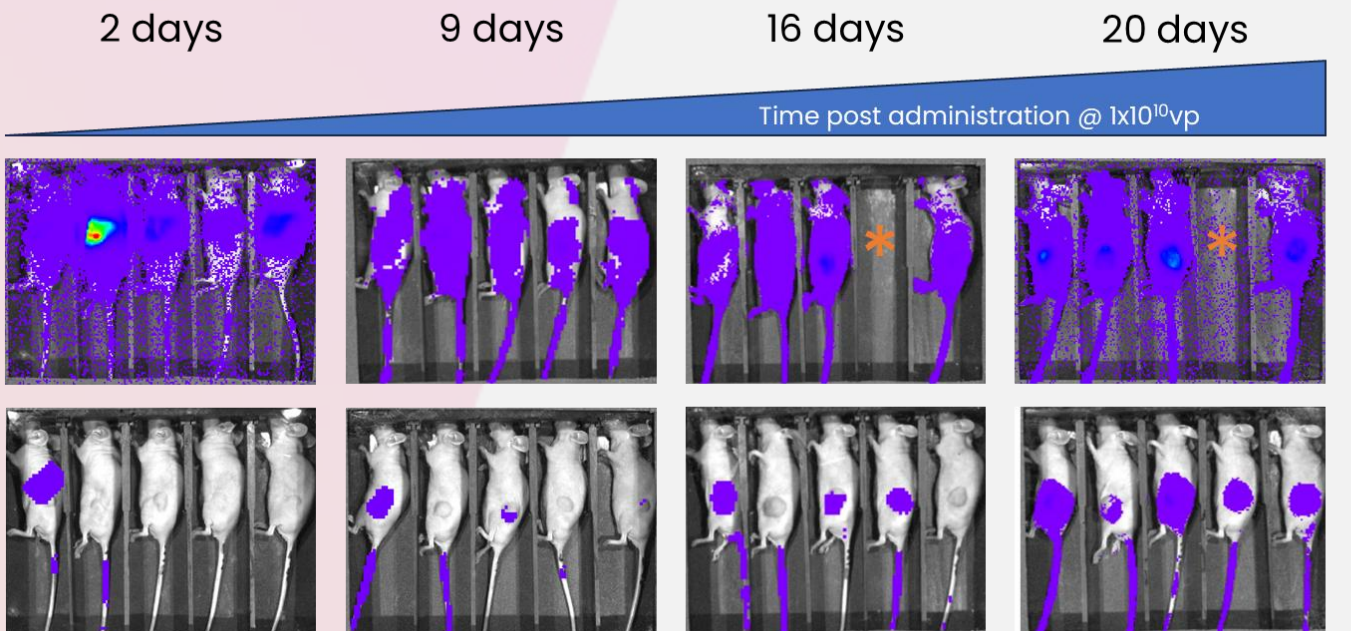


Figure 6: Luciferase transgene activity detected by IVIS in SKOV3 ovarian tumor xenografts on the flank of BALB/c nude mice at day 2, 9, 16 or 20 post single intravenous administration with 1×10^{10} vp/animal of unmodified Ad5 (top row) or TROCEPT (bottom panel) expressing a luciferase transgene. n=5/group, the same mice are shown in the same order at each timepoint. * sacrificed due to tumor burden.

7. Phase 1 clinical study (ATTEST) is open and recruiting

A phase 1 study, ATTEST, is evaluating safety and preliminary efficacy of intravenous delivery of ATTR-01 in patients with selected epithelial tumours expressing high levels of the target $\alpha\text{v}\beta 6$ integrin, including non-small cell lung, urothelial, head and neck, pancreatic, endometrial and cholangiocarcinoma in multiple sites in UK.

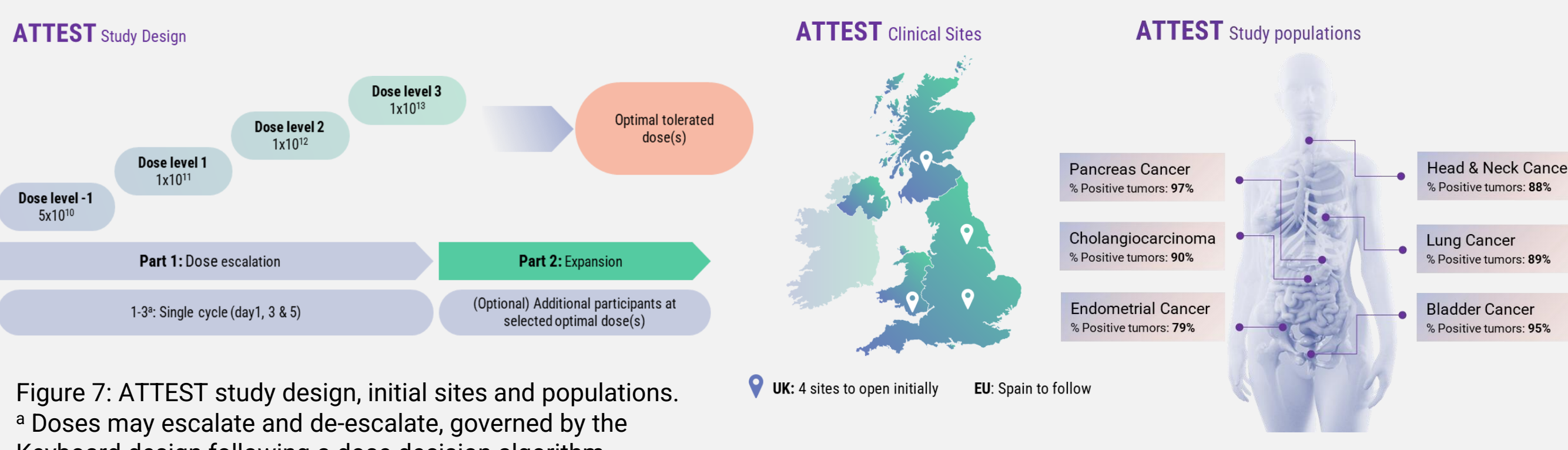


Figure 7: ATTEST study design, initial sites and populations. * Doses may escalate and de-escalate, governed by the Keyboard design following a dose decision algorithm.

8. TROCEPT: a precision virotherapy platform

TROCEPT is a first-in-class platform for expression of immuno-oncology therapeutic transgenes to tumors.

TROCEPT avoids liver uptake – one of the major limitations of other systemically delivered viral therapies.

TROCEPT encoding an anti-PD-L1 antibody (called ATTR-01) is being testing in a Ph1 clinical study (ATTEST) in pancreatic, lung, head and neck, cholangiocarcinoma, endometrial and bladder cancers.

TROCEPT: Delivering therapeutic transgenes **Multiple partnering opportunities**

