



Supplementary Materials for

Cell Carriage, Delivery, and Selective Replication of an Oncolytic Virus in Tumor in Patients

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Fig. S2. Reoviral RNA can also be detected in patient PBMCs, granulocytes, and platelets using an alternative, second set of primers.

Table S1. Histological data summary for all patients.

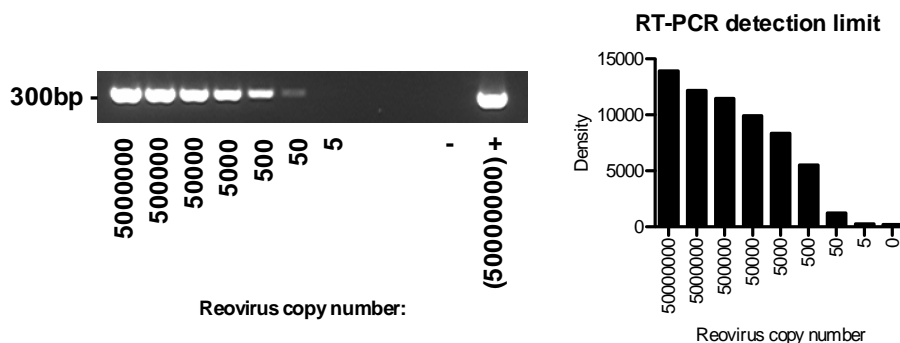
Table S2. Coexpression of reovirus and tubulin in tumor.

SUPPLEMENTARY MATERIALS AND METHODS

RNA detection limits. RNA was extracted from 4.6×10^9 TCID₅₀/ml reovirus stocks using the QIAamp Viral Mini Kit (Qiagen), which equates to $\sim 5 \times 10^7$ reoviral copies entering the RT-PCR reaction. 10 fold dilutions were made and amplified accordingly using the OneStep RT-PCR Kit (Qiagen). Reovirus sigma 3 cDNA targeted primers (Sigma-Aldrich Ltd.) used were: forward 5'-GGGCTGCACATTACCACTGA and reverse 5'-CTCCTCGCAATACAACCTCGT - a detection limit of 35 cycles was used for evaluation. Samples were run on a 2% agarose gel and analyzed for reovirus RNA by the presence of a 300bp PCR product. A negative (RNase-free water) control was included. Bands were analyzed for densitometry using ImageJ software.

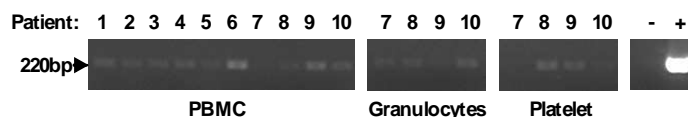
RNA detection using an alternative, second set of primers. RNA was extracted from 7×10^5 PBMC, granulocytes and platelets using the QIAamp Viral Mini Kit and amplified using the OneStep RT-PCR Kit as described before. Reovirus cDNA targeted primers (Sigma) used were: forward 5'-TGCAGCGTGAAGAGTCCATA and reverse 5'-ATGCACTTAGCTGCGGACTT - a detection limit of 35 cycles was used for evaluation. Samples were run on a 2% agarose gel and analyzed for reovirus RNA by the presence of a 220bp PCR product. Positive (reovirus RNA) and negative (RNase-free water) controls were included.

FIGURE S1



Reoviral RNA is detectable when ~50 copies enter the reaction, but not ~5 copies or fewer. RNA was extracted from reovirus stocks so that $\sim 5 \times 10^7$ reoviral copies entered the RT-PCR reaction. 10 fold dilutions of the RNA were made to assess the detection limit of RT-PCR at 35 cycles (left). A negative (RNase-free water) control was included and bands were analyzed for densitometry (right).

FIGURE S2



Reoviral RNA can also be detected in patient PBMCs, granulocytes, and platelets using an alternative, second set of primers. Data show 1 hour post 1st infusion samples assessed directly for reoviral RNA by RT-PCR. Granulocyte and platelet samples were unavailable for patients 1-6. Reovirus RNA and RNase-free water were included as positive and negative controls.

TABLE S1

| Patient | Tumor reovirus staining | Liver reovirus staining | Tumor reovirus EM | Liver reovirus EM | Reovirus/caspase co-localization (% positive cells) |
|---------|-------------------------|-------------------------|-------------------|-------------------|---|
| 1 | weak | negative | positive | negative | NA |
| 2 | negative | negative | negative | negative | 0 |
| 3 | weak | negative | positive | negative | NA |
| 4 | weak | faint | positive | negative | 5 |
| 5 | strong | faint | positive | positive | NA |
| 6 | strong | faint | positive | positive | NA |
| 7 | strong | negative | positive | positive | 10 |
| 8 | strong | faint | positive | positive | 5 |
| 9 | strong | negative | positive | negative | 30 |
| 10 | strong | faint | positive | positive | 10 |

Histological data summary for all patients. Tumor and liver were assessed for the presence of reovirus sigma 3 capsid protein by electron microscopy (EM) and reovirus sigma 3 capsid protein (+/- caspase-3 co-expression) by immunohistochemistry (IHC). Note that electron microscopy reovirus findings correlated with immunohistochemistry in all cases, except for patient 4 liver (faint on IHC; negative on EM) and patient 7 liver (negative on IHC; positive on EM). These inconsistencies are likely due to the different sensitivities of the two techniques and/or sampling discrepancies. NA denotes samples that were unavailable for analysis.

TABLE S2

| Patient | Reovirus/tubulin co-localization (% positive cells) |
|---------|---|
| 1 | 0 |
| 2 | 0 |
| 4 | 15 |
| 7 | 40 |
| 8 | 30 |
| 10 | 40 |

Coexpression of reovirus and tubulin in tumor. In 6 assessable patients (patients with adequate available tissue), tumor was assessed for the presence of reovirus sigma 3 capsid protein and its co-expression with tubulin.