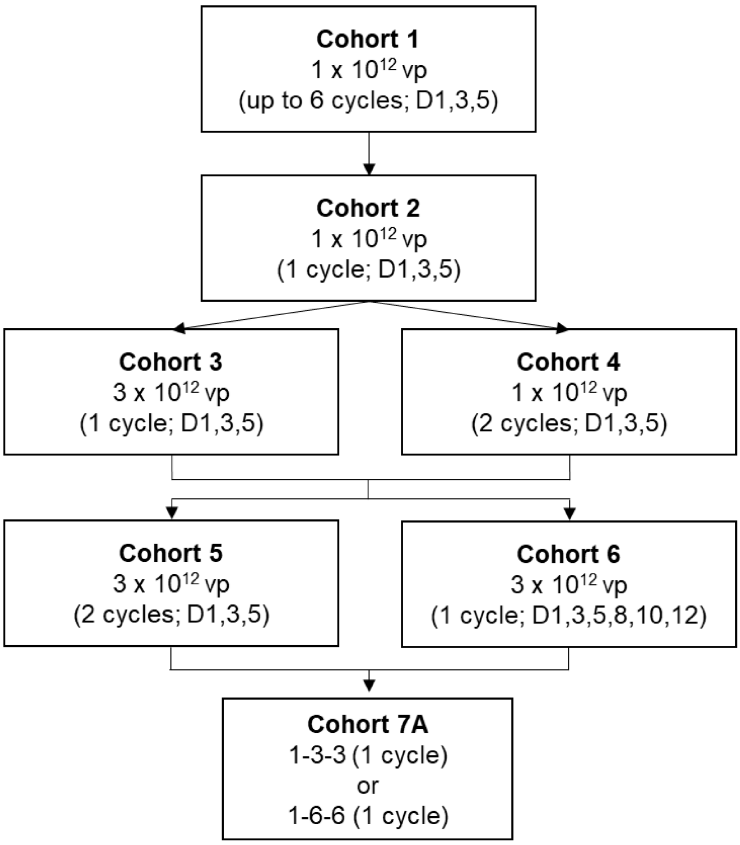
## Supplementary materials

### Methods

Study design

Supplementary figure 1. Study design




1-3-3=1 x $10^{12}$ vp on Day 1 followed by 3 x $10^{12}$ vp on Days 3 and 5; 1-6-6=1 x $10^{12}$ vp on Day 1 followed by 6 x $10^{12}$ vp on Days 3 and 5.

In Cohort 1, patients received enadenotucirev in combination with pembrolizumab (up to 6 cycles); in Cohorts 2 to 7A, patients received enadenotucirev in combination with nivolumab (up to 8 cycles)

Histopathology and immunohistochemistry

PanCK-CD8 stained slides were evaluated by a pathologist using a density proportion score algorithm. The output of this analysis was the relative surface area (%) of the tumor with a marker-positive immune cell density that belonged to one of the 8 density bins. The density

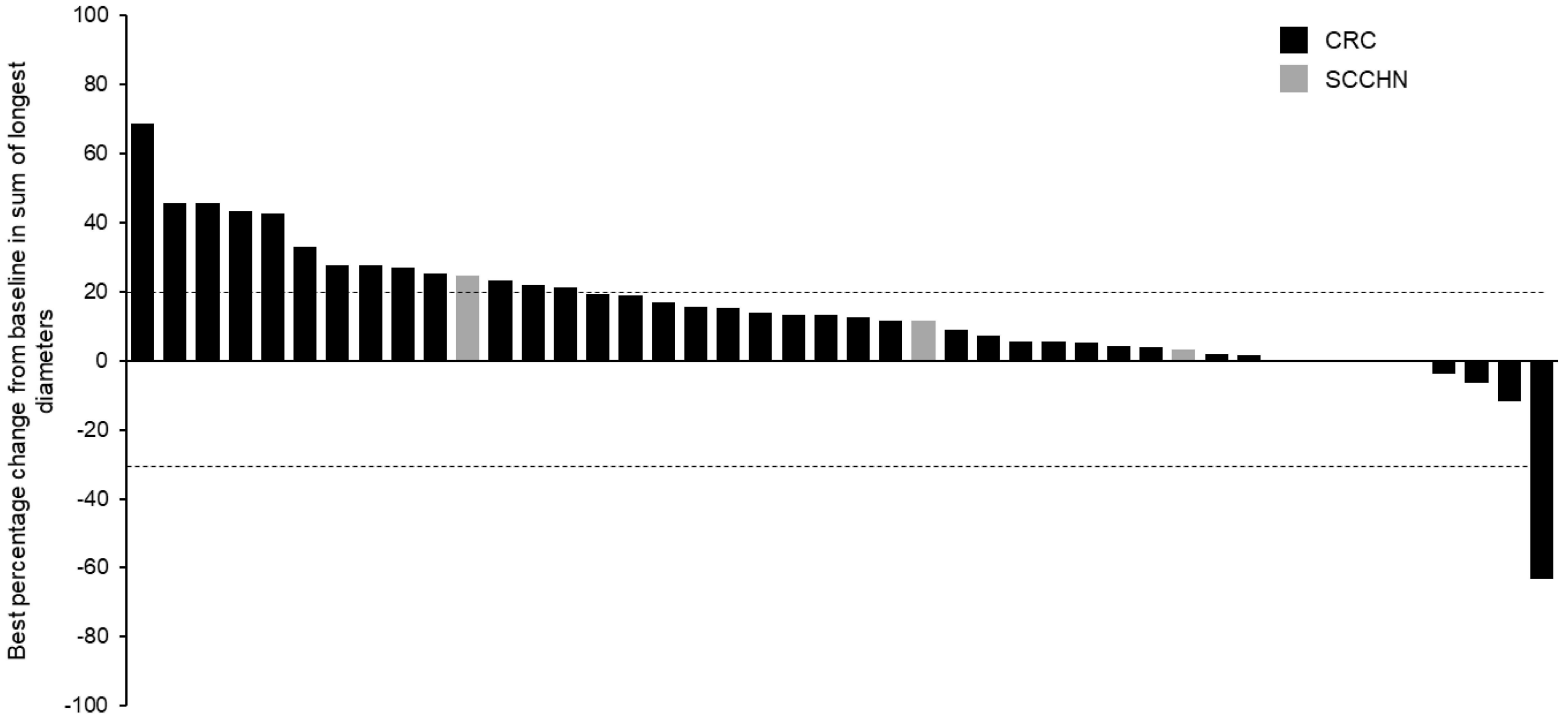
bins were linked to marker-specific reference images that were used by the pathologist. There were 4 density bins for intra-stromal positive immune cells (ITS0-ITS3, with ITS0 having the least CD8+ cells and ITS3 the most) and 4 density bins for intra-epithelial positive immune cells (IE0-IE3, with IE0 having the least CD8+ cells and IE3 the most). Density of CD8-positive cells was measured separately in both stromal and epithelial compartments. The immune phenotype was scored as ‘desert’, ‘inflamed’, ‘excluded’ in the IHC-stained slides using the following criteria:

- Desert: high IE0+IE1 and ITS0+ITS1≥80%
- Excluded: IE0+IE1≥80% and ITS0+ITS1<80%
- Inflamed: IE2+IE3≥20%

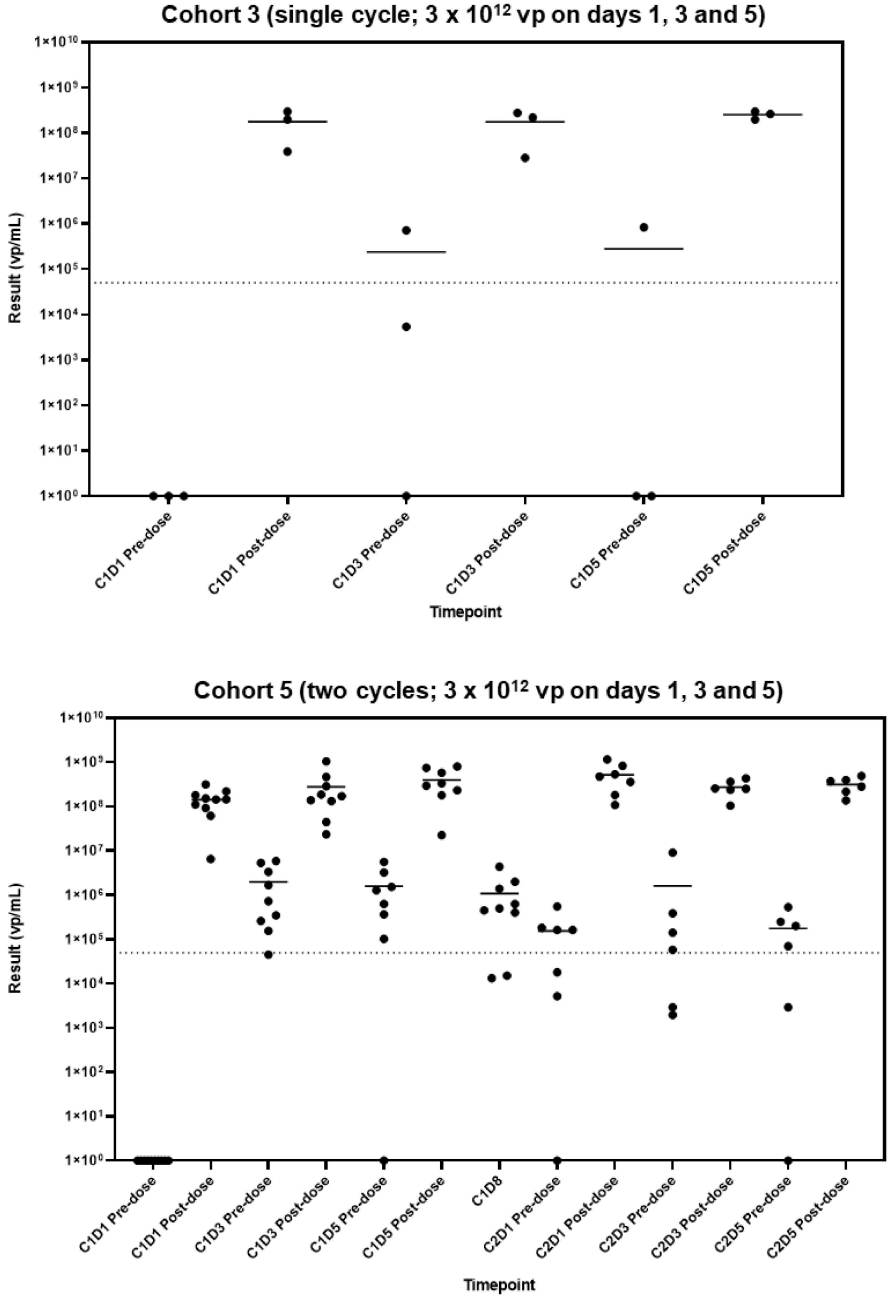
If the positive immune cell spread was too heterogenous, the immune phenotype was scored as ‘heterogenous’.

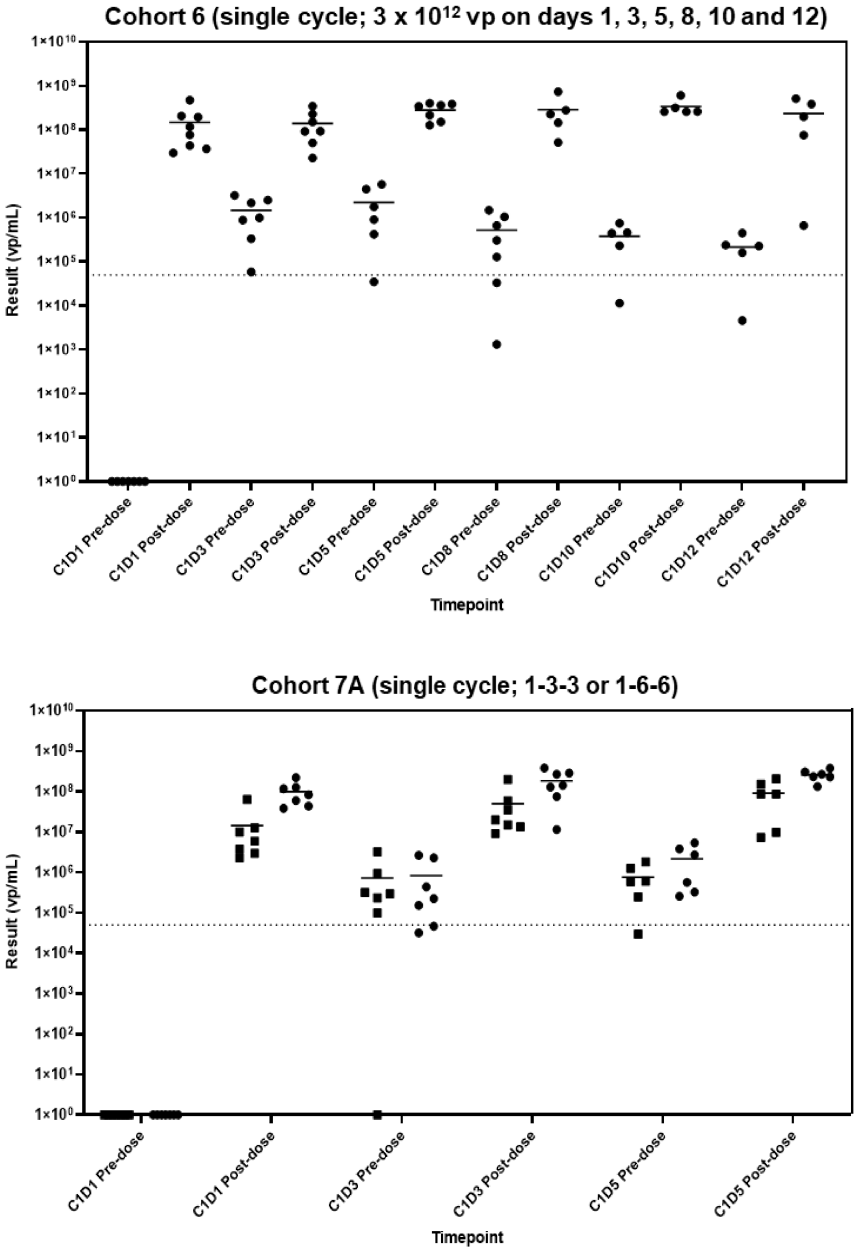
**Results**

Supplementary figure 2. Best change in target lesion burden (full analysis set)



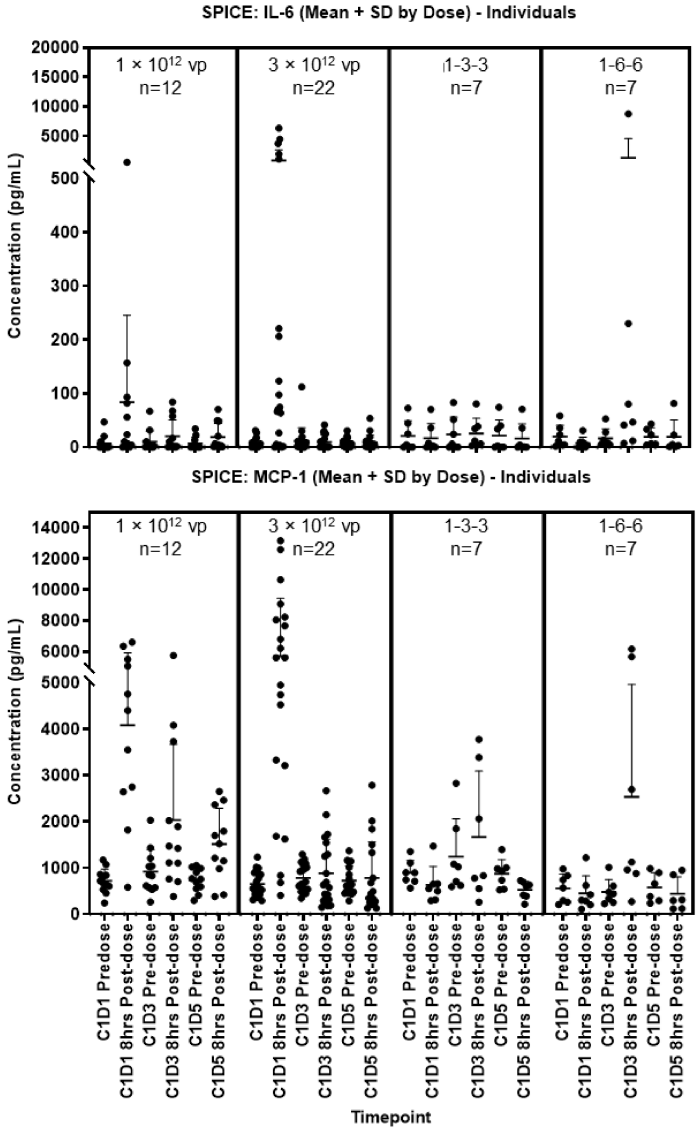
Supplementary figure 3. Pharmacokinetics in cohorts receiving enadenotucirev at a dose of 3 x $10^{12}$ vp



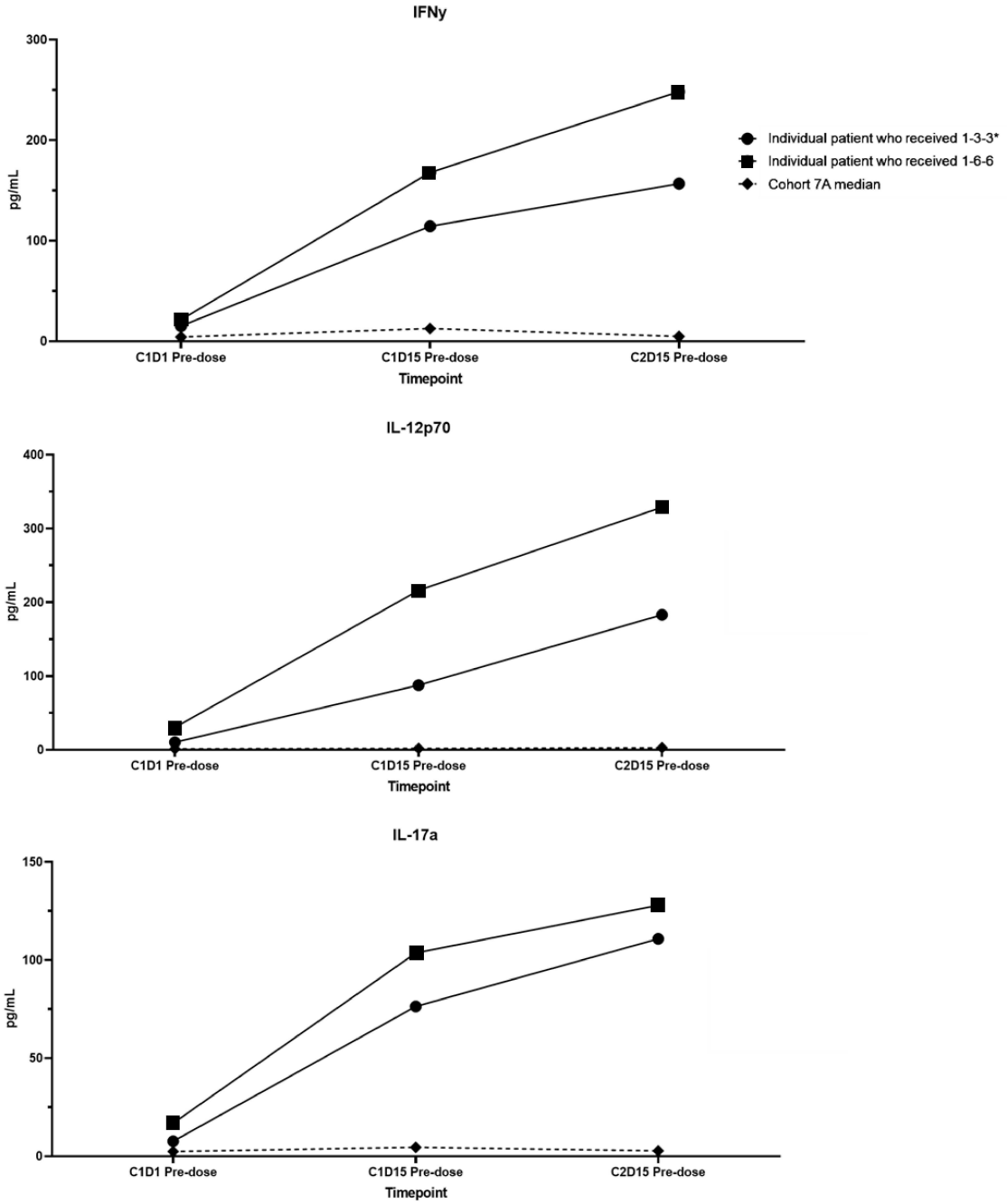



Data points represent individual patients; solid horizontal lines represent the mean value; dashed line indicates the limit of quantification. Values below the limit of quantification (LOQ; 5 x $10^4$ vp/mL for the cohorts shown above) are included for information purposes in order to demonstrate persistence of the virus. Although the concentration values below the LOQ cannot be guaranteed to be as reliable as those above the LOQ, the $C_T$ values obtained below the LOQ in the qPCR assay are considered reliable in detecting virus.

Supplementary figure 4. Acute (Day 1-5) cytokine responses



Supplementary figure 5. Increases in Th1 and related cytokines in Cohort 7A




*patient achieved PR