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Effect of brincidofovir on adenovirus and A549 cells transcriptome profiles.

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Running title: Effect of BCV on adenovirus and cellular expression.

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ABSTRACT

Objectives.

Human adenovirus (HAdV) infections are associated with a high morbidity and mortality in transplant patients requiring the use of antiviral treatments. Brincidofovir (BCV), a cytidine analog, inhibits HAdV replication through viral DNA elongation termination and likely through other mechanisms. To elucidate if BCV regulates cellular antiviral pathways, we analyzed its impact on HAdV-infected and non-HAdV-infected lung epithelial cells.

Methods.

We assessed the cellular and viral transcriptome of A549 cells infected and non-infected with HAdV C5 and treated or non-treated with BCV by RNAseq after 72 hours.

Results.

BCV treatment of HAdV infected cells resulted in a profound decrease of viral transcription associated with a relative overexpression of the early genes E1A and E4 and of the late gene L1. BCV had also a profound impact on A549 cells' transcriptome. Ontologic analysis revealed an effect of BCV on several pathways known to interact with adenovirus replication as mTor signalling and Wnt pathways. A549 cells treated with BCV demonstrated a significant inhibition of the biological function of "viral replication" including 25 dysregulated genes involved in inflammation pathways.

Conclusion.

We demonstrated that BCV alters viral gene expression and promotes the expression of antiviral cellular pathways in A549 cells. These results provide new insights how to interfere with cellular pathways to control HAdV infections.

INTRODUCTION

Human adenoviruses (HAdV) are non-enveloped viruses with linear double-stranded DNA genome. There are currently 103 types of HAdV divided into seven species (A to G) (“HAdV Working Group,” n.d.). HAdV are responsible for many diseases that are often benign in immunocompetent individuals. In hematopoietic stem cell allogeneic transplant patients (HSCT), especially children, HAdV are associated with a high morbidity and mortality. In case of a disseminated infection, the mortality rises to more than 50%(Lion, 2014).

No antiviral drug has been validated for the treatment in humans yet. However, some drugs are used out of FDA or EMA approval, including cidofovir or brincidofovir (BCV). Cidofovir (CDV) is an acyclic nucleoside phosphonate. Its active form, cidofovir-diphosphate (CDV-PP) competitively inhibits incorporation of deoxycytidine into viral DNA, leading to viral DNA termination (Magee et al., 2005; Xiong et al., 1997). CDV is nephrotoxic (Ljungman et al., 2003), has poor cellular uptake and demonstrates a weak tissue distribution. Thus, CDV does not reach enough plasma concentrations with usual dosages to allow efficient control of HAdV viremia in absence of immune recovery (Heemskerk et al., 2005; Lugthart et al., 2015).

BCV, which was recently developed, is a lipid conjugate of CDV with a greater oral bioavailability, no nephrotoxicity and a greater intracellular delivery of the active antiviral, CDV-PP. Previous studies have shown that BCV is active against Adenovirus, Herpesvirus and Orthopoxvirus with a lower half maximal effective concentration (EC50) than cidofovir (Lanier et al., 2010). BCV has demonstrated *in vitro* activity on several types of HAdV (Hartline et al., 2005; Toth et al., 2008).

Both BCV and CDV have been reported to possess antiviral activity also against DNA viruses that do not encode their own polymerase such as Polyomaviruses, Papillomaviruses or Parvoviruses (Bua et al., 2019; Tylden et al., 2015). For those viruses, the exact mechanism of inhibition has not been yet established. Inhibition of helicase activity has been suggested for Polyomaviruses and Papillomaviruses (Andrei et al., 2015; Bernhoff et al., 2008).

Other reports suggested that CDV may induce epithelial apoptosis (Detweiler et al., 2018), cellular DNA damage and aberrant mitoses (Verhees et al., 2019) or participate in antiangiogenic effects in infected cells (Amine et al., 2006). The impact of CDV seems also to be dependent on the replicative or latent status of the virus. In addition to the direct action of the nucleotide analog, the lipid moiety may also contribute to the antiviral activity of BCV. Indeed a study evaluating the inhibition of Ebola virus replication by BCV showed the antiviral activity required its lipid moiety (McMullan et al., 2016).

These data suggest that CDV or BCV may present other mechanisms than direct viral inhibition by triggering cellular pathways involved in viral control or cell death. In order to gain insight into the cellular and viral response to BCV treatment and potentially identify new targets involved in the control of HAdV infection, we investigated the viral and cellular transcriptome of A549 cells infected with HAdV and treated with BCV by RNAseq technology.

MATERIAL AND METHODS

Drug, cell culture and virus infection

A549 human lung adenocarcinoma cells were cultured in DMEM with 10% foetal bovine serum (FBS), 100U/mL penicillin and 100ug/mL streptomycin under

standard conditions at 37°C in 5% CO₂ humidified air. 350,000 A549 cells/well were seeded in six well tissue culture plates for an 80% confluence the following day. The cells were then washed twice with phosphate-buffered saline and infected with HAdV C5 ATCC (VR-5) at MOI of 0.003 or mock using DMEM medium for one hour at 37°C. Afterward, the inoculum was removed and the cells were washed with PBS then treated with or without BCV (1.2 µM) in DMEM 2 % of FBS.

RNA isolation

After 72 hours, the cells were washed three times with PBS and then lysed in 1mL of RNeasy Protect Cell Reagent (Qiagen, Hilden, Germany) with 200µL of DMEM.

100µL of cell suspension was withdrawn for DNA extraction. The suspension was centrifuged for five minutes at 5,000G and the supernatant was removed. RNA was extracted with RNeasy Plus Mini Kit (Qiagen) following the manufacturer's instructions. The total RNA quality was assessed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, California) and RIN score greater than eight were considered acceptable. Total RNA quantity was determined with Qubit RNA HS assay (Thermo Fisher Scientific Waltham, Massachusetts).

DNA isolation and HAdV DNA quantification.

DNA was extracted from 100µL of cell suspension with NucliSENS easyMAG (bioMérieux, Marcy-l'Étoile, France) with specific B protocol and eluted in 40µL. HAdV DNA was quantified with the Adenovirus R-gene kit (bioMérieux),

according to the manufacturer's instructions. The assays were performed using an ABI 7500.

Library preparation and sequencing

cDNA libraries were prepared from 0.5µg of total RNA using the TruSeq RNA Library Prep Kit v2 (Illumina, SanDiego, California) according to manufacturer's instructions. Prior to sequencing, libraries were quantified with kappa library quantification kit (Roche, Bâle, Swiss) and quality was assessed with High Sensitivity RNA ScreenTape on a 2200 TapeStation system (Agilent Technologies). All libraries were pooled and sequencing was performed on Nextseq 550 (Illumina) with a 75x2 cartridge.

Bioinformatic analysis

Conversion and demultiplexing of reads were performed using bcl2fastq. FastQC was used for quality controls of the raw data. Reads were cleaned using the Trimmomatic software with a minimum quality threshold of Q30 (Bolger et al., 2014). Cleaned reads were aligned and counted to the annotated *Homo sapiens* genome (GRCh38.p11) and the annotated HAdV C5 genome (accession number AY339865) using STAR software (Dobin et al., 2013). Principal component analysis and identification of differentially expressed genes were performed using the package DESeq2 (Love et al., 2014). Genes with an absolute fold change greater than two and an adjusted *p*-value less than 0.05 (FDR < 5 %) were considered to be differentially expressed. Ontologic analyses were performed with IPA software (Qiagen). In addition to determining the enriched biological pathways for each comparison (Fisher's exact test, *p*-value <0.05), for each

enriched biological pathway, the software calculate a Z score. The Z score determines whether the changes in gene expression for the known genes of each biological pathway are consistent with what is expected from the literature (Z score positive) or if the changes are inversely correlated with the literature (Z score negative).

RESULTS

Descriptive statistics of RNAseq data

To obtain a broad picture of the A549 cells response to infection with HAdV and BCV treatment, RNAseq was used to analyse differential gene expression at the mRNA level. In total, there were four experimental conditions including mock-infected lung epithelial cells (condition A549, n=2), A549 cells infected with C5 ATCC HAdV (condition ADV, n=3), A549 cells infected with C5 ATCC HAdV with BCV treatment (condition ADV+BCV, n=3), and mock-infected A549 cells with BCV treatment (condition BCV, n=3). Read counts for every sample is detailed in Table 1. A median of 94,642,847 reads per sample were sequenced. For each sample, 92 to 98 percent of reads were mapped on human genome. In total, more than 20,000 human genes covered by sequencing have been analysed for each condition.

Brincidofovir's effect on viral transcription

We analyzed the viral transcriptome to evaluate the impact of BCV on HAdV gene expression. Principal component analysis (PCA) showed that all the replicates of the two conditions ADV and ADV/BCV clustered within their respective condition (Figure 1A). The BCV treatment significantly decreased viral

transcription. On average only 0.02 percent of the reads were aligned with the HAdV genome in cells treated with BCV compared to 3.08 percent for cells infected with HAdV but not treated with BCV ($p=0.0252$). Quantification of HAdV DNA corroborates these results with a significant lower mean of HAdV DNA levels ($1.74E+07$ copies/ng of genomic DNA) in HAdV infected cells treated with BCV than in infected cells without BCV treatment ($2.73E+09$ copies/ng of genomic DNA) ($p=0.0142$).

The coverage map of read transcript counts within the adenovirus genome showed that late transcripts (L3, L4 and L5) were the most abundant at 72 hours post-infection in untreated condition. The decrease of HAdV transcripts in HAdV infected cells treated with BCV was not linear over the entire HAdV genome. Indeed, after normalization of the number of reads aligned, BCV treatment was associated with an overexpression (fold change > 2 , $FDR < 5\%$) of the early genes E1A 32kDa protein and E4 and of the late gene L1 compared to infected A549 cells without BCV. BCV treatment was also associated with a decreased expression (fold change < -2 , $FDR < 5\%$) of the late genes L2 pV, L3 PVI, L4 100K, pVIII and L5 PVI and of the early gene E3 12.5K (Figures 1B and 2).

Brincidofovir induces gene expression modification in adenovirus infected and non-infected A549 cells

We performed an unsupervised analysis among all samples with a PCA on cellular transcript read counts. This analysis showed that each condition has a distinct transcriptome profile (Figure 3A). BCV treatment appeared to be the main cause of transcriptome alteration as indicated along PC1 (86% of variance versus 8% for PC2). In order to observe the transcriptional changes caused by

HAdV infection and BCV treatment, we identified the differentially expressed (DE) genes (fold change > |2|, FDR < 5%) between 3 comparisons: ADV vs A549, BCV vs A549 and ADVBCV vs ADV (Figure 3B). The highest level of differential expression was found in ADVBCV vs ADV comparison with 555 DE genes (480 downregulated and 75 upregulated). A total of 374 and 113 DE genes were identified in ADV vs A549 and BCV vs A549 comparisons, respectively. The overlap of all three comparisons together found only four DE genes (FTH1, MRPL12, NPTX1 and PRR7) (Figure 3C). The largest overlap of DE genes (218) was found between ADVBCV vs ADV and BCV vs A549 comparisons (Figure 3 C). A more precise view of the overlap between the DE genes of the three comparisons is available in Supplementary Figure 1. The comparison of the impact of BCV on HAdV infected (BCV vs ADV) and non-infected A549 cells (BCV vs A549) showed 218 DE genes in the same direction (207 upregulated and 11 downregulated). On the contrary, most of DE genes that overlapped between ADV vs A549 and ADVBCV vs ADV comparison had the opposite expression (67 genes downregulated in ADVBCV vs ADV and upregulated in ADV vs A549 comparison). Finally, ADV vs A549 and BCV vs A549 comparisons have few common DE genes, only 9 genes with parallel expression and one with an opposite expression. These data suggest that BCV modified the cellular response to adenovirus infection in infected cells without having an opposite cellular transcriptional profile to that of HAdV infection in non-infected cells.

Brincidofovir Induces Regulation of Multiple Cellular Pathways in Infected and Uninfected Cells

We investigated the effect of BCV treatment on biological cell pathways. Significantly enriched biological pathways ($p < 0.05$) of BCV vs A549 and ADVBCV vs ADV comparisons with a predicted inhibition or activation (non-null Z score) are shown in Figures 4A and B. All pathways that were significantly enriched with a null Z score value were listed in Supplementary Table 1.

In non-infected A549 cells (BCV vs A549), BCV increased the sirtuin signaling pathway that is involved in a wide range of cellular processes such as transcription, apoptosis and inflammation. On the contrary, BCV had a negative effect on the Wnt and PCP signalling pathways. The mTOR signaling pathway was also inhibited by BCV treatment, an interesting feature as HAdV proteins E4-ORF1 and E4-ORF4 activate mTOR (O'Shea et al., 2005).

We looked at the biological pathways enriched during BCV treatment of HAdV infected cells (ADVBCV vs ADV). The Wnt and PCP signalling pathways remained inhibited despite HAdV infection while the impact on the sirtuin signaling pathway disappeared. Other pathways were also inhibited, such as the biosynthesis of heparan sulfate, the STAT3 pathway and the NER pathway. Activated pathways included PTEN signalling involved in cell cycle regulation and LXR/ RXR activation, which regulates the inflammatory response of macrophages.

Brincidofovir induces gene expression modifications involved in viral replication in uninfected cells

A comparative analysis of the biological functions significantly enriched with an absolute Z score greater than one is represented in Figure 5. The heatmap representation enables an overall view of the different biological pathways for

each of the three comparisons. The biological functions concerning viral replication ("replication of Influenza virus", "replication of influenza A virus", "replication of virus" and "replication of RNA virus") were found to be significantly enriched with a negative z-score with a BCV treatment in non-infected cells (BCV vs A549), suggesting that BCV enables inhibition of viral replication also through the regulation of cell transcription.

Other biological functions were predicted to be activated only in A549 treated with BCV only (BCV vs A549) such as "release of prostaglandin E2" and "transcription". The function of the "accumulation of tumor cell lines" corresponding to tumor cell lines proliferation was enriched with a positive z-score by BCV in both infected and non-infected cells.

The activation of functions "synthesis of ceramide", "synthesis of sphingolipid" and "metabolism of sphingolipid" were predicted in HAdV infected cells treated with BCV compared to those without BCV (ADVBCV vs ADV).

While there was no effect of BCV in non-infected cells on intracellular transport, BCV treatment of infected cells (ADVBCV vs ADV) was associated with a control of the biological functions "microtubule dynamics", "organization of cytoplasm" and "organization of Cytoskeleton" that were enriched upon HAdV infection.

Analysis of the DE genes by brincidofovir involved in the inhibition of viral replication in uninfected cells.

In non-infected cells, 25 DE genes were involved in the inhibition of the viral replication by BCV. A heatmap of the differential expression of the 25 genes among the three comparisons is shown in Figure 6A. In HAdV infected cells, fourteen genes (56 percent) remained DE by BCV, thus confirming the strong

impact of BCV on cell transcription and its involvement in cellular antiviral effect. The 25 DE genes were subjected to an ontological analysis in order to specify the biological pathways involved (Figure 6B) as well as the upstream regulators responsible for modulating the transcription of these genes (Figure 6C). Several biological pathways were found to be significantly enriched, such as "interferon signalling" (IFITM1, ISG15), fatty acid biosynthesis (FASN), "IL10 signalling" (HMOX1, IL1B), "LXR / RXR activation" (L1B / SFASN), "STAT3 pathway", "NFK-B signalling" (FGFR2, IL1B), "role recognition pattern recognition in bacteria and viruses" or "CD42 signalling" (APC2, TNK2). The putative regulators of the 25 DE genes identified with highest p-value were NOS2, the cytokine TNFSF10, the PI3K family involved in the Wnt pathway, calcitriol and the STAT3 transcription regulator.

DISCUSSION

In the present study, we identified that BCV induced cellular expression involved in the regulation of viral infection that may participate, in complement of its direct action of the viral polymerase inhibition, in the control of HAdV infection. Our results provide new insights on cell pathways that could be targeted to enhance the inhibition of HAdV replication.

In this study, we analyzed the impact of HAdV infection and BCV treatment at 72 hours post-infection and post-treatment in A549 cells. Although HAdV infection has a strong impact on cellular transcription a few hours after infection, the regulation of cellular expression mediated by cidofovir takes more time and is

poorly measurable within 48 hours of treatment in cell lines tested so far. In cervical carcinoma cell lines positive for human papillomavirus, after 24 hours of CDV exposure, only two genes were downregulated and no gene was upregulated, while a total of 27 and 140 genes were DE after 48 hours and 72 hours of CDV exposure, respectively (De Schutter et al., 2013). Based on this previous observation, we decided to assess the transcriptome profiles in A549 cells at 72 hours to be able to evaluate both direct and indirect antiviral acting effects of BCV.

With the analysis of RNAseq of cellular transcripts, we were able to assess the impact of BCV treatment on HAdV transcription. At 72 hours following HAdV infection without BCV treatment, structural genes including L3, L4 and L5 were the most detected. Our results are consistent with previous reports looking at HAdV gene expression between zero to 72 hours. Expression of late genes was reported to start at 12 hours with a peak of expression reached 48 hours post-infection (Crisostomo et al., 2019; Flint and Sharp, 1976). HAdV infected cells treated with BCV showed a dramatic decrease of both viral DNA and viral RNA levels.

The analysis of the relative abundance of HAdV transcripts revealed that BCV treatment was associated with an over expression of early genes E1A, and E4 and the late gene L1. Inhibition of viral DNA replication and late protein expression may enable persistent expression of early genes. However, there is no data about a negative feedback of viral DNA on expression of early genes. A relative overexpression of L1 transcripts is consistent as L1 region is transcribed during the early phase and before viral DNA replication occurs (Farley et al.,

2004). BCV treatment was also associated with a decreased expression of the late genes L2 pV, L3 PVI, L4 100K and pVIII and L5 PVI. A drop in late gene transcripts was expected as the activation of the late major promoter depends on the viral genome replication (Thomas and Mathews, 1980; Ziff and Evans, 1978). Finally, a decrease of the early protein E3-12.5K was observed with BCV. The majority of proteins encoded in the E3 region are involved in the modulation the host immune response, either to prevent the cellular presentation of viral proteins or to inhibit tumor necrosis factor-mediated cytotoxicity. Whether the decrease of E3-12.5K may facilitate recognition and/or lysis of HAdV infected cells deserves further investigation.

We found also that BCV had a strong impact on cellular transcription both in non-infected cells and HAdV infected cells, with most of the DE genes being downregulated. Those changes could help to understand clinical side effects. BCV has been associated with gastrointestinal symptoms in hematopoietic stem cell transplantation (HSCT) patients, including abdominal pain, diarrhea, nausea, and vomiting (Marty et al., 2019). We found that BCV inhibited the Wnt signaling pathway, which is critical for development and renewal of the digestive epithelial cells (Mah et al., 2016). This could support an intestinal toxicity of BCV, although this should be interpreted cautiously as we used a respiratory cell line.

Ontologic analysis revealed in non-infected A549 cells a BCV mediated downregulation on several biologic pathways, including some known to interact with adenovirus replication, such as mTOR signalling, and Wnt pathway (O'Shea et al., 2005; Zhao et al., 2007). Two HAdV proteins, E4 ORF1 and E4 ORF4 activate mTOR for promoting viral replication (O'Shea et al., 2005). Wnt

signaling is a powerful regulator of cell proliferation and differentiation. It has been shown that the modulation of this pathway can alter the replication of certain viruses such as HCMV (Kapoor et al., 2013). A study has reported that Wnt pathway was activated during adenovirus infection, although the underlying mechanism remains undetermined (Zhao et al., 2007). Other pathways, such as the sirtuin signalling pathway, were not previously described in adenovirus infection but are known to interact with several others DNA and RNA viruses (Budayeva et al., 2016). Indeed, sirtuins regulate gene expression primarily by controlling the modification status of histones and transcription factors. The transcriptional functions of certain sirtuins appear necessary for the regulation of viral gene expression. Altogether these results suggest that BCV in non-infected cells interfere with several pathways involved in the regulation of viral expression.

In HAdV infected cells, BCV treatment was associated with an upregulation of sphingolipid and ceramide synthesis, that have been associated previously with the propagation or the inhibition of various viruses, and a downregulation of pathways involved in intracellular transport. Whether such alterations may participate in the control of HAdV replication needs further investigation.

Specifically, we found that BCV treatment of non-infected cells induced an inhibition of the “viral replication” function. The 25 genes involved in this inhibition are involved in inflammation pathways such as interferon and IL10 signalling, LXR/RXR activation, STAT3 pathway or NF- κ B signalling. Interestingly, one of the putative regulators found by an upstream analysis of these 25 genes is the PI3K family. The HAdV E4 ORF1 protein is known to

interact with PI3K resulting in mTor signalling pathway activation and increased viral replication (Kong et al., 2015; O'Shea et al., 2005).

The inhibition of the PI3K family and mTor signalling pathway may thus play a role in the anti-HAdV action of BCV. The key role of E4 ORF1 as E4 ORF4 in HAdV cycle is highlighted by the findings of their relative overexpression after HAdV DNA inhibition by BCV. Thus, their indirect inhibition by BCV may help for the control of HAdV infection.

BCV includes the nucleotide analog CDV and a lipid conjugate. Whether one part or the other is responsible for the indirect antiviral action remains also to be elucidated. Indeed the lipid moiety was found to be required for antiviral properties against Ebola virus (McMullan et al., 2016), while CDV did not show any action against this RNA virus. Few studies are available on the effect of CDV on the cellular transcriptome. A study by De Schutter *et al* focused on the effect of CDV at 72 hours post treatment on keratinocyte lineages (De Schutter et al., 2013). They showed an effect on the inflammatory response, and activation of the pathways Rho GTPase and LXR/RXR. Activation of the LXR/ RXR pathway was also found in our study in infected cells. On the other hand, some pathways found to be enriched by BCV treatment of uninfected cells in our study were not mentioned in the work of De Schutter *et al.*, such as mTor signaling, Wnt pathway, PCP pathway or Sirtuin signaling pathway.

Our work was conducted in A549 cell lines, often used to assess antiviral properties of various compounds. Other cell lines and primary cells should be tested to confirm indirect antiviral properties of BCV.

BCV has been tested in randomized clinical trials for its efficacy to control disseminated infections. A few reports of compassionate treatments have also been published. For most of the direct acting antiviral drugs, failures are usually consecutive to the selection of resistance mutations in targets genes. Some mutations in the HAdV DNA polymerase have been associated *in vitro* with resistance to BCV and CDV. BCV treatment failures were observed in both clinical trials and case reports but only one case was reported to be associated with a mutation of resistance to BCV (V303I) (Grimley et al., n.d.). Therapeutic failures in absence of viral resistance could be explained by a reduced indirect action of BCV because of host or viral genetic diversity. Indeed, a recent study suggested that the genetic diversity in the E4 region may influence HAdV virulence (Dhingra et al., 2019).

In conclusion, our work shows that BCV, in A549 cells, regulates cellular expression involved in the control of viral replication, including pathways that interact with HAdV early coding genes, in particular PI3K family and mTor signalling pathways. Further research is needed to understand better the cellular interactome of BCV and its importance in the treatment of viral infections.

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TRANSPARENCY DECLARATION

None to declare.

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Table 1. Statistics of RNAseq data and mapped reads

Sample	Raw Reads	% of reads aligned on GRch38	% of reads aligned on HAdV C5
A549-1	83531044	98%	0.00%
A549-2	57858128	97%	0.00%
ADV-1	76811456	96%	1.44%
ADV-2	95207058	94%	3.35%
ADV-3	120216508	92%	4.44%
BCV-1	140581864	97%	0.00%
BCV-2	50302032	98%	0.01%
BCV-3	160939108	97%	0.00%
ADVBCV-1	94078636	97%	0.02%
ADVBCV-2	111150208	97%	0.02%
ADVBCV-3	147258948	97%	0.02%

Legends for figures

Figure 1. Global overview of brincidofovir effect on human mastadenovirus

C5 transcriptome

A) PCA plots of replicates from ADV and ADVBCV conditions. The percentages on each axis represent the percentages of variation explained by the principal components. B) Heatmap of adenovirus C5 genes. Each row of the heatmap represents the z-score transformed $\log_2(1 + \text{FPKM})$ values of one differentially expressed gene across all samples (blue, low expression; red, high expression).

Figure 2. Coverage map of differentially expressed genes of adenovirus. Grey genes are not found significantly differentially expressed by brincidofovir treatment, blue genes were downregulated by brincidofovir treatment and red genes were upregulated by brincidofovir treatment.

Figure 3. Global overview of cellular RNAseq data

A) PCA plots of samples from the four experimental conditions. The percentages on each axis represent the percentages of variation explained by the principal components. B) Summary of differentially expressed genes ($|\log_2\text{-fold change}| > 1$, $\text{FDR} < 5\%$) for three comparisons of interest: ADV vs A549, BCV vs A549 and ADVBCV vs ADV. C) Venn Diagram showing the total number of overlapping DE genes profile for the three comparisons.

Figure 4. Significantly enriched biological pathways by brincidofovir in infected and uninfected cells

IPA identified pathways ($P \leq 0.05$) that are activated or inhibited ($|z\text{-score}| > 0$) in differentially expressed genes from BCV vs A549 (A) and ADVBCV vs ADV comparison (B). $\log_{10}(\text{P-value})$ is reported on the upper axis, with the threshold P-value indicated by a red line at $-\log_{10}(\text{P-value}) = 1.3$. Enriched pathways with a positive Z_score are in orange font, those with a negative Z score are in blue.

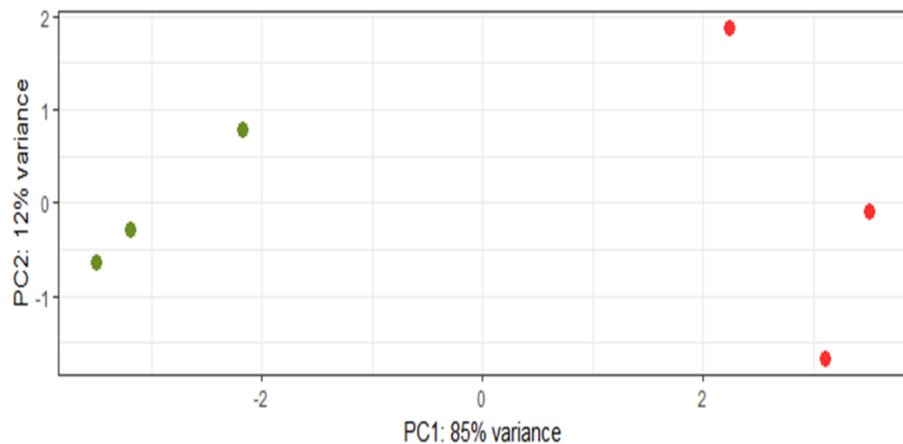
Figure 5: Heatmap comparing diseases and biological functions of interest significantly enriched among the three comparisons.

IPA identified diseases and biological functions significantly enriched ($P \leq 0.05$) that are activated or inhibited ($|z\text{-score}| > 0$) in differentially expressed genes from BCV vs A549, ADV vs A549 and ADVBCV vs ADV comparisons. Enriched pathways with a positive z-score are in orange font, those with a negative z-score are in blue

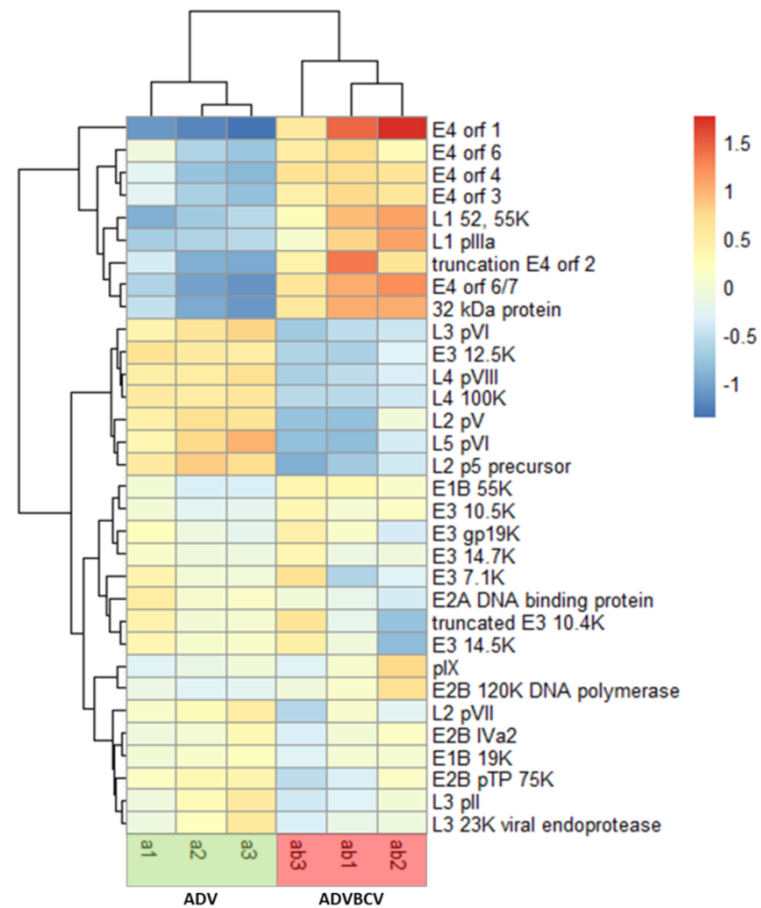
Figure 6 : Analysis of the 25 differentially expressed genes by brincidofovir involved in the inhibition of viral replication in uninfected cells.

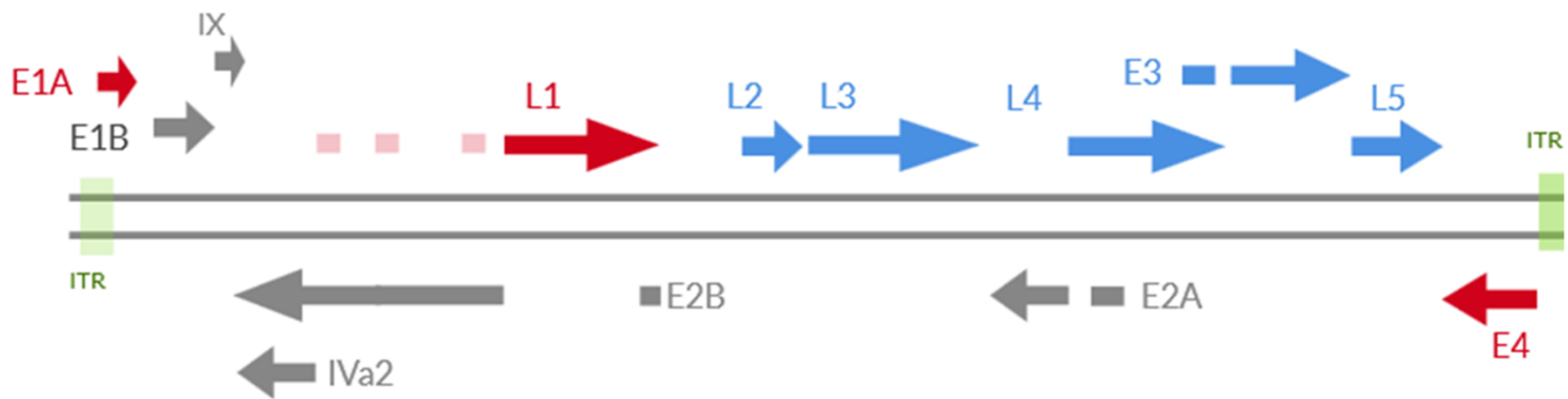
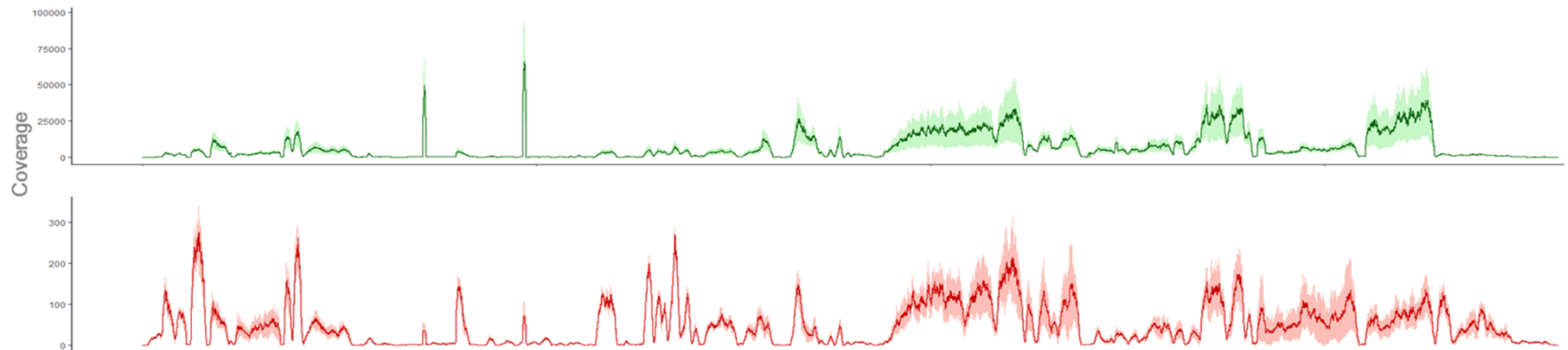
A) Heatmap of the expression of the 25 genes in the three comparisons. Genes upregulated are in red font and those downregulated in green font. B) Top 15 significantly enriched biological pathways by the 25 genes. IPA identified significantly enriched pathways ($p < 0.05$). $\log_{10}(P\text{-value})$ is reported on the upper axis. C) Top 15 upstream regulators of the 25 genes. IPA identified significantly enriched putative upstream regulators ($p < 0.05$). $\log_{10}(P\text{-value})$ is reported on the upper axis.

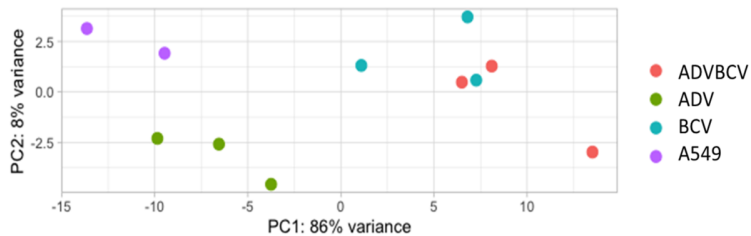
A



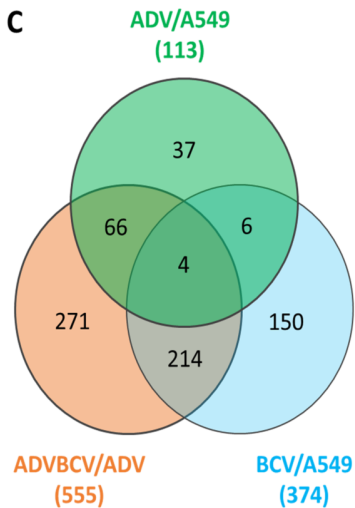
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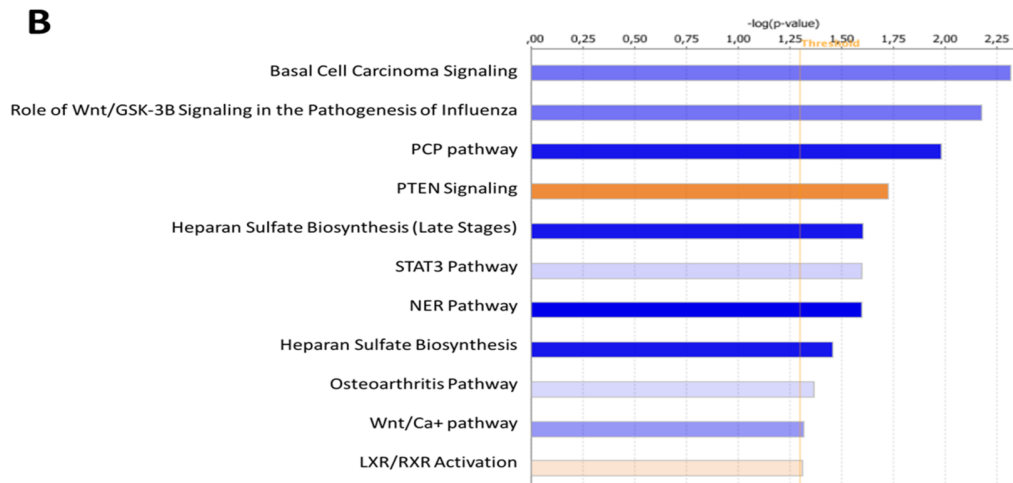
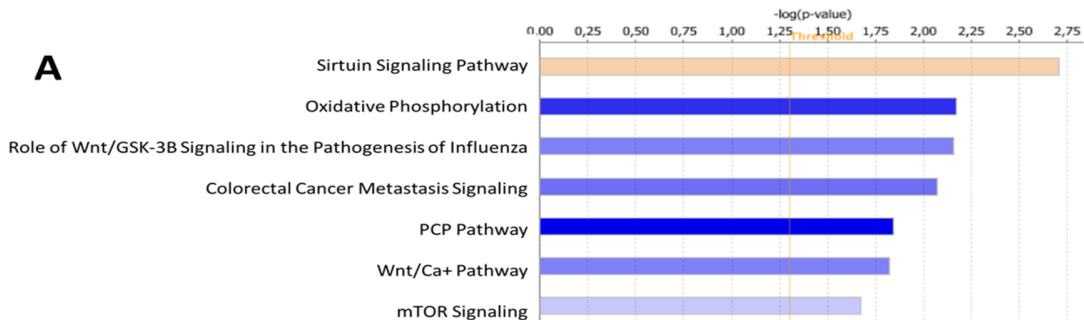


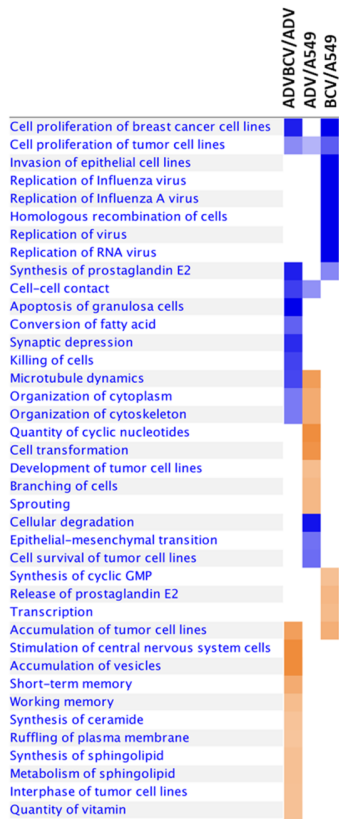


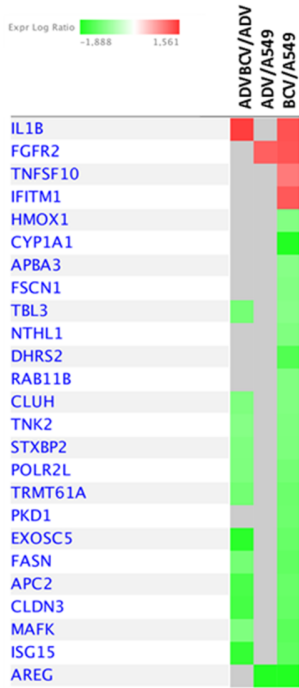
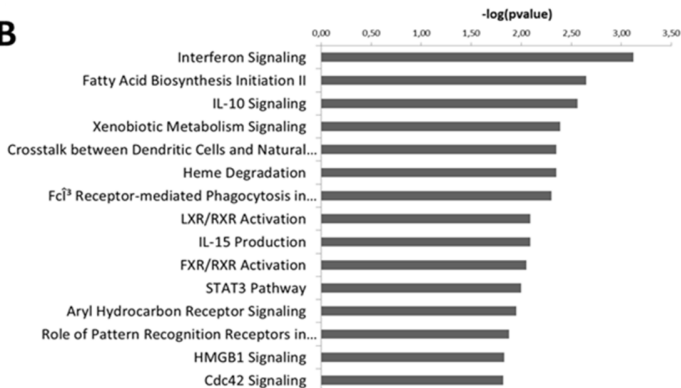
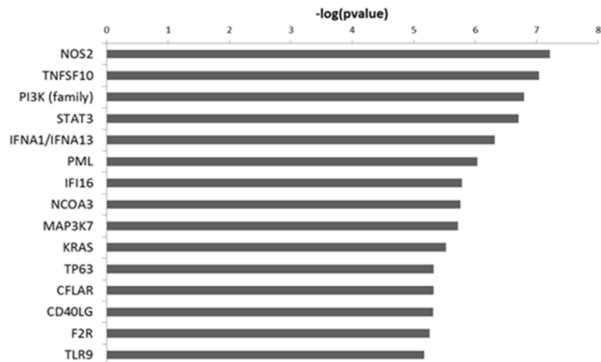
A**B**

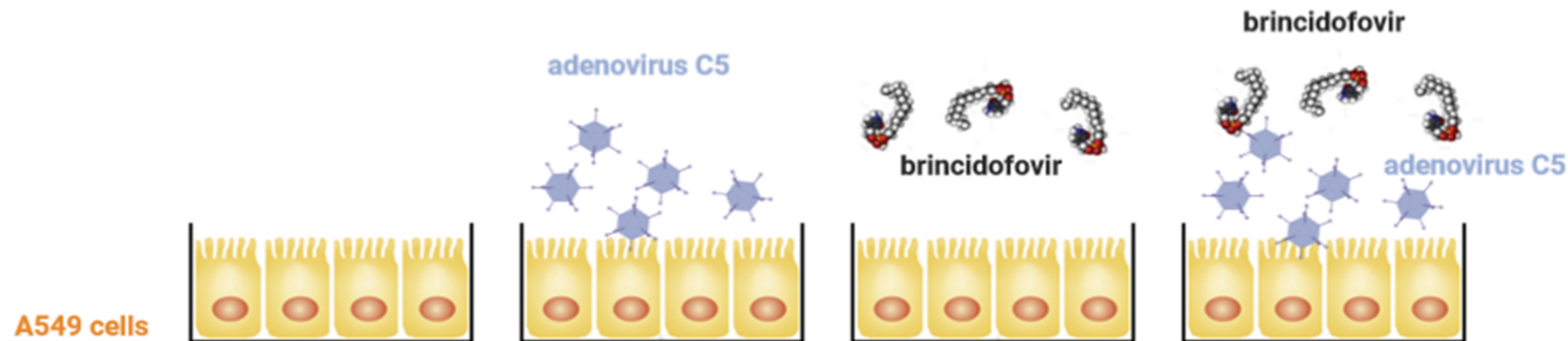
	Down-regulated genes	Up-regulated genes
ADVvsA549	13	100
BCVvsA549	330	44
ADVBCVvsADV	480	75

C





A**B****C**



Viral and Cellular Transcriptom analysis

