PARVOVIRUS

Dzelila Jusic
Haris Rudalija
Tarik Sidran
Introduction

• Family: parvoviridae (parvovirinae and densovirinae)
• *Parvus* (Latin) = small
• Hosts: mammals, birds, insects
• *Virion* > icosahedral; 18-26 nm; ss DNA 4-6 Kb; linear
Dependoviruses

• Parvovirinae subfamily
• Defective, need helper virus to co-infect the cell
• Observed as contaminant in adenovirus preparation
Dependoviruses

- Gene vectors
Autonomous parvoviruses

• Do not need helper virus!
• B19 (from family densovirinae) infects red blood cell precursors
• Fifth disease

Figure 12.2 Child with fifth disease. Reproduced by permission of the New Zealand Dermatological Society.
Other diseases caused by B19

- Acute arthritis
- Aplastic anaemia
- Hydrops foetalis
Some other paroviruses discovered

- Genus *Bocavirus*
- Other viruses from subfamily *Densovirus*
- *Bombyx mori*

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Parvovirinae</em></td>
<td><em>Dependovirus</em></td>
<td>Adeno-associated virus 2</td>
</tr>
<tr>
<td></td>
<td><em>Parvovirus</em></td>
<td>Minute virus of mice</td>
</tr>
<tr>
<td></td>
<td><em>Erythrovirus</em></td>
<td>Feline panleukopenia virus</td>
</tr>
<tr>
<td></td>
<td><em>Bocavirus</em></td>
<td>B19 virus</td>
</tr>
<tr>
<td></td>
<td><em>Iteravirus</em></td>
<td>Human bocavirus</td>
</tr>
<tr>
<td><em>Densovirinae</em></td>
<td></td>
<td><em>Bombyx mori</em> densovirus</td>
</tr>
</tbody>
</table>
Parvovirus virion

- Small virus; simple structure; ssDNA genome;
- Capsid: icosahedral symmetry
- 60 protein molecules
- VP1: the largest protein
- Virion has surface protrusions and canyons
GENOME

• ss DNA
• ITR’s (inverted terminal repeats)
• Parvovirus replication>> restricted only to the S phase
• Attachment and entry >> virion enters in the cell by endocytosis>> transported to the nucleus>> passes through nuclear pore
• ssDNA to dsDNA
TRANSCRIPTION AND TRANSLATION

• RNA polymerase II transcribes the virus genes
  • cell’s transcription factors are crucial
  • various splicing events of pre-mRNA
  • two sizes classes of mRNA:
    larger mRNA – non-structural proteins – phosphorylated – control of gene expression in replication
    smaller mRNA – structural proteins
DNA REPLICATION

• Paroviruses special for DNA replication by rolling-hairpin replication (leading strand mechanism)
• Formation of procapsids
• Loading of procapsids with + or – ssDNA
• dsDNA unwound by helicase (non-structural protein)
DEPENDOVIRUS REPLICATION

• Phenomenon of tandem work of dependovirus and helper virus causes productive infection with both viruses

• If dependovirus infect a cell alone, then the virus genome may be integrated as dsDNA into a cell chromosome

• Recombination of two DNAs and latent infection

• In humans integration at site in chrom 19
Other ssDNA viruses

- Majority of known ssDNA viruses have circular genomes
- Parvoviruses are the only known viruses with ssDNA linear genomes
Oncolytic H1-Parvovirus Shows Safety and Signs of Immunogenic Activity in a First Phase I/IIa Glioblastoma Trial
What is this paper all about?

- Improving prognosis of malignant brain tumors through usage of rat H-1PV
- H-1 parvovirus suppresses tumors through oncolysis and stimulation of anticancer immune response
- ParvOryx01 - the first phase I/IIa clinical trial
- H-1PV injected intratumorally and intravenously
- 9 days after treatment, virus re-administered around tumor resection cavity
Primary endpoints of the trial:

• Safety
• Tolerability
• Virus distribution
• Maximum tolerated dose (MTD)
• Progression-free and overall survival
• Levels of viral and immunological markers in the tumor and peripheral blood
Achievements of the trial:

- H-1PV treatment is safe and well tolerated
- No MTD was reached
- Virus crosses blood-brain/tumor barrier and spreads widely
- Favorable pharmacokinetics
- Induced antibody formation (dose-dependent)
- Specific T cell responses triggered
- H-1PV may trigger and immunogenic stimulus
- Median survival extended in comparison with meta-analyses
- Further H-1PV clinical development desirable
INTRODUCTION

• Glioblastoma is the most aggressive primary human brain tumor

• Research is conducted because median survival is in the range of only 13-15 months at first diagnosis and 6-9 months at recurrence :( 

• Novel approach like oncolytic virotherapy being investigated with HPV, adenovirus, reovirus, prodrug-converting enzyme retrovirus etc.
RESULTS (Patients and Treatment)

- Eighteen patients (mean age: 57.8 ± 10.6 years) enrolled in ParvOryx01
- They are chosen based on strict key eligibility criteria and pre-trial requirements are given
- They are tested on some mutations and tumor size is determined
- The 18 patients were assigned to two treatment arms differing in the mode of first virus application
<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Treatment Group</th>
<th>Dose (PFU)</th>
<th>Previous Therapies</th>
<th>MGMT Methylation</th>
<th>IDH1 Mutation</th>
<th>Cross-Sectional Area (mm²)</th>
<th>KPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-01</td>
<td>51</td>
<td>male</td>
<td>G1-L1</td>
<td>1E6</td>
<td>S, RAD, TMZ</td>
<td>ND</td>
<td>neg</td>
<td>112</td>
<td>100</td>
</tr>
<tr>
<td>1-02</td>
<td>42</td>
<td>male</td>
<td>G1-L1</td>
<td>1E6</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>108</td>
<td>80</td>
</tr>
<tr>
<td>1-03</td>
<td>62</td>
<td>male</td>
<td>G1-L1</td>
<td>1E6</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>266</td>
<td>100</td>
</tr>
<tr>
<td>2-04</td>
<td>70</td>
<td>male</td>
<td>G1-L2</td>
<td>5E7</td>
<td>S, RAD, TMZ</td>
<td>NA</td>
<td>neg</td>
<td>288</td>
<td>100</td>
</tr>
<tr>
<td>2-05</td>
<td>53</td>
<td>female</td>
<td>G1-L2</td>
<td>5E7</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>3,300</td>
<td>100</td>
</tr>
<tr>
<td>2-06</td>
<td>64</td>
<td>male</td>
<td>G1-L2</td>
<td>5E7</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>2,772</td>
<td>80</td>
</tr>
<tr>
<td>3-07</td>
<td>48</td>
<td>female</td>
<td>G1-L3</td>
<td>1E9</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>805</td>
<td>80</td>
</tr>
<tr>
<td>3-08</td>
<td>44</td>
<td>male</td>
<td>G1-L3</td>
<td>1E9</td>
<td>S, RAD, BEV, IRI</td>
<td>no</td>
<td>neg</td>
<td>731</td>
<td>100</td>
</tr>
<tr>
<td>3-09</td>
<td>45</td>
<td>male</td>
<td>G1-L3</td>
<td>1E9</td>
<td>S, RAD, BEV, IRI</td>
<td>no</td>
<td>neg</td>
<td>638</td>
<td>70</td>
</tr>
<tr>
<td>4-10</td>
<td>69</td>
<td>male</td>
<td>G2-L2</td>
<td>5E7</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>1,925</td>
<td>60</td>
</tr>
<tr>
<td>4-11</td>
<td>47</td>
<td>male</td>
<td>G2-L2</td>
<td>5E7</td>
<td>S, RAD, BEV, IRI</td>
<td>no</td>
<td>neg</td>
<td>629</td>
<td>100</td>
</tr>
<tr>
<td>4-12</td>
<td>64</td>
<td>male</td>
<td>G2-L2</td>
<td>5E7</td>
<td>S, RAD, TMZ</td>
<td>NA</td>
<td>NA</td>
<td>770</td>
<td>90</td>
</tr>
<tr>
<td>5-13</td>
<td>66</td>
<td>male</td>
<td>G2-L3</td>
<td>1E9</td>
<td>S, RAD, TMZ</td>
<td>ND</td>
<td>neg</td>
<td>1,519</td>
<td>90</td>
</tr>
<tr>
<td>5-14</td>
<td>52</td>
<td>male</td>
<td>G2-L3</td>
<td>1E9</td>
<td>S, RAD, TMZ</td>
<td>yes</td>
<td>neg</td>
<td>336</td>
<td>90</td>
</tr>
<tr>
<td>5-15</td>
<td>55</td>
<td>female</td>
<td>G2-L3</td>
<td>1E9</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>1,056</td>
<td>100</td>
</tr>
<tr>
<td>6-16</td>
<td>62</td>
<td>female</td>
<td>G3-L4</td>
<td>5E9</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>575</td>
<td>90</td>
</tr>
<tr>
<td>6-17</td>
<td>76</td>
<td>male</td>
<td>G3-L4</td>
<td>5E9</td>
<td>S, RAD, TMZ</td>
<td>yes</td>
<td>neg</td>
<td>2,184</td>
<td>100</td>
</tr>
<tr>
<td>6-18</td>
<td>71</td>
<td>male</td>
<td>G3-L4</td>
<td>5E9</td>
<td>S, RAD, TMZ</td>
<td>no</td>
<td>neg</td>
<td>1,881</td>
<td>90</td>
</tr>
</tbody>
</table>

BEV, bevacizumab; IDH1, isocitrate dehydrogenase 1; IRI, irinotecan; KPS, Karnofsky performance status; MGMT, O^{6}-methylguanine-DNA methyltransferase; NA, not available; ND, not determinable; neg, negative; PFU, plaque-forming units; RAD, radiation therapy; S, surgery; TMZ, temozolomide.
A

- Screened: n=23
- Not enrolled: n=5
  - Complete tumor resection not possible (n=1)
  - Tumor regression (n=2)
  - Pathological ECG (n=1)
  - Unable to give informed consent (n=1)

Enrolled: n=18

- Group 1, Level 1: N=3
- Group 1, Level 2: N=3
- Group 1, Level 3: N=3
- Group 2, Level 2: N=3
- Group 2, Level 3: N=3
- Group 3, Level 4: N=3

No exclusions

B

- G1-L1, G1-L2, G1-L3 (total doses: 1E6, 5E7, 1E9 PFU) & G3-L4 (total dose: 5E9 PFU)
  - Single intratumoral administration of ParvOryx
  - Surgery and administration of ParvOryx into the wall of the resection cavity

Study Days: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

- G2-L2, G2-L3 (total doses: 5E7, 1E9 PFU)
  - Five intravenous infusions of ParvOryx
  - Surgery and administration of ParvOryx into the wall of the resection cavity

Study Days: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
TREATMENT TOLERANCE

• ParvOryx treatment showed:
  • No dose-limiting toxicity (DLT)
  • No impact on safety laboratory parameters
  • No changes in electrocardiogram or vital signs
  • 13 adverse events (1 unrelated to ParvOryx, 12 classified as "serious", required hospitalization, life-threatening or medically relevant)
  • Patient 6-16(G3-L4) met the criteria of SUSAR (suspected unexpected serious adverse reaction) and after 6 months life support was suspended on request of the family
  • The event wasn't considered as a DLT due to unproven casualty, trial was continued
### Table 2. Individual Clinical Responses in All 18 Patients

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Subject ID</th>
<th>Progression-free Survival (PFS)(^a)</th>
<th>Overall Survival (OS)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days(^c)</td>
<td>Direct Documentation(^d)</td>
</tr>
<tr>
<td>G1-L1</td>
<td>1-01</td>
<td>171</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>1-02</td>
<td>18</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>1-03</td>
<td>170</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>2-04</td>
<td>161</td>
<td>no</td>
</tr>
<tr>
<td>G1-L2</td>
<td>2-05</td>
<td>19</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>2-06</td>
<td>15</td>
<td>yes</td>
</tr>
<tr>
<td>G1-L3</td>
<td>3-07</td>
<td>111</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>3-08</td>
<td>119</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>3-09</td>
<td>53</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>4-10</td>
<td>55</td>
<td>no</td>
</tr>
<tr>
<td>G2-L2</td>
<td>4-11</td>
<td>28</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>4-12</td>
<td>169</td>
<td>no</td>
</tr>
<tr>
<td>G2-L3</td>
<td>5-13</td>
<td>17</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>5-14</td>
<td>111</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>5-15</td>
<td>112</td>
<td>yes</td>
</tr>
<tr>
<td>G3-L4</td>
<td>6-16</td>
<td>46</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>6-17</td>
<td>56</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>6-18</td>
<td>19</td>
<td>yes</td>
</tr>
</tbody>
</table>

\(^a\)According to the trial protocol, the study visits could take place within a 2-week interval before or after the respective dates. Therefore, the values of the individual PFS may slightly vary from the predetermined ones.

\(^b\)Whenever applicable, patients were followed up for OS beyond the regular study follow-up period of 6 months by means of telephone interviews or visits to the trial center. Therefore, the timing of actual censoring for individual OS may exceed 6 months.

\(^c\)PFS, days after surgery; OS, days after first administration of ParvOryx.

\(^d\)PFS, progressive disease documented by trial-specific investigations (MR scans) versus third-party communication; OS, date of death known versus censoring at end of the study.
Pharmacokinetics

- Blood concentrations of H-1PV viral genomes (Vg) and infectious particles measured
- Determination of systemic virus availability
- H-1PV can cross the blood-brain/tumor barrier in a dose-dependent manner also in humans
VIRUS TRANSMISSION FROM STUDY PATIENTS TO THIRD PERSONS: RISK ASSESSMENT

- Samples of feces, urine and saliva tested for Vg presence
- Intratumorally treated patients> fecal H1-PV excretion at highest virus dose
- All but one intravenously treated patients> were positive at even lower doses
- No Vg in feces beyond 20 days
VIRUS TRANSMISSION FROM STUDY PATIENTS TO THIRD PERSONS: RISK ASSESSMENT

• In urine> Vg present in only G2-L3 patients at low conc.
• All saliva samples> negative
• This rules out the risk of virus transmission
H1-PV VIRUS EXPRESSION IN TUMOR TISSUE

• Virus suspended in Ringer solution >> possible to visualize initial distribution of ParvOryx after injection by CT

• Observation proved that: SLOW INJECTION (1mL in 30 min) is optimal to keep virus in tumor tissue >> no backflow to catheter
H1-PV VIRUS EXPRESSION IN TUMOR TISSUE

- H1-PV presence in resected tumor tissue by FISH
- Viral DNA revealed in 11 out of 12 intratumorally treated patients
- Positive FISH signals appeared also at catheter- distant tumor areas>>meaningful penetration of ParvOryx into tumor tissue
CATHEPSIN B INDUCTION AFTER LOCAL PARVORYX ADMINISTRATION

• Expression of Cathepsin B (CTS B) examined in resected tumor tissue
• Further analysis of interaction between glioblastoma cells and ParvOryx
• All patients showed CTSB induction (left figure) in contrast to past negative controls (right figure)
CATHEPSIN B INDUCTION AFTER LOCAL PARVORYX ADMINISTRATION

• Majority of CTSB- overexpressing cells identified as MICROGLIA/ MACROPHAGE

• Also, CTSB positive non-macrophage cells detected but overexpressing EGFR

• Intravenously treated patients >> CTSB expression lower
INFILTRATION OF TUMORS WITH ACTIVATED IMMUNE CELLS

• Prominent immune cell infiltrates present in ParvOryx treated patients
• TIL’s >>CD 45
• T-lymphocytes specific CD3 marker
• B lymphocytes not detected, NK cells scarce
• CD8 and CD4 (positive T lymphocytes) >>two major subpopulations
INFILTRATION OF TUMORS WITH ACTIVATED IMMUNE CELLS

- T cell activation assessed by granzyme B and perforin staining
INfiltration of tumors with activated immune cells

IFN-γ

IL-2
INfiltration of tumors with activated immune cells

- Interferon and Interleukin-2 are markers that indicate T cell cytotoxic potential.
- In situ, FOX P3 analysis revealed only few regulatory T (Treg) cells, not concentrated in main immune infiltrate.
SPECIFIC T-CELL RESPONSES IN THE PERIPHERAL BLOOD OF PARVORYX-TREATED PATIENTS

• By IFN and ELISpot assays>>examination of virus inducted specific cellular response> meassuring reactivity of T cells to viral antigenic determinants.

• 9 of 12 patients showed significant T-cell response against antigens introduced (N1 and VP)

• Virus- reactive T –cells were found at all patients and persisted around several months.
SPECIFIC T-CELL RESPONSES IN THE PERIPHERAL BLOOD OF PARVORYX-TREATED PATIENTS

• Further investigation of activated lymphocyte specificity by testing shorter and single viral peptides>> identification of specific cytotoxic T-lymphocyte epitopes.

• Significant T cell response to Glioma antigens, detected also
• ParvOryx01 was a first-in-human trial for the use of H-1PV in recurrent glioblastoma patients. Despite disparities within the trial population that were expected from the rather wide inclusion criteria regarding gender, age, tumor size, and previous treatments, ParvOryx was generally well tolerated over the entire range of investigated doses. Thus, the primary objective of safety and tolerability was met. There were no signs of systemic inflammation, excessive immune activation, or main organ toxicity.

• ParvOryx01 demonstrated for the first time in humans the ability of H-1PV to pass, in a dose-dependent manner, from a brain tumor to the bloodstream and vice versa. This confirms preclinical findings in rats, showing systemic availability of the virus after intracerebral injection.

• Our analysis of the H-1PV distribution after local injection demonstrates that a single, slow injection through a standard catheter resulted in excellent targeting of the inoculum to the tumor area and wide distribution of H-1PV through the tumor tissue. Future clinical trials using ParvOryx for this or other tumor types might thus avoid exploring more complicated methods for local administration such as convection-enhanced delivery.
Figure 4. In Situ Analysis of Tumors Resected after Local ParvOryx Administration (A–E) Intratumoral virus replication and host inflammatory reaction (patient 6-17). (A and B) H-1PV transcripts (A) and NS1 proteins (B) were detected in virus-injected tumor tissue (left), but not in historical controls (right). (C) Double staining was performed for (left) viral RNA (red) and glial fibrillary acidic protein (green), or (right) viral NS1 (red) and epidermal growth factor receptor (green). (D) H-1PV-transcript-accumulating tumor cells (red) stained negative for the macrophage marker CD68 (green) (left). In contrast, the majority of cathepsin B (CTSB)-positive cells (red) expressed CD68 (green) (right). CTSB+/CD68_ cells were also detected (arrow). (E) Increased CTSB expression was observed in ParvOryx-treated tumor (left), as compared with historical control (right). (F–I) Tumor infiltration with activated immune cells (patient 6-16). (F) Upper two panels: the treated tumor showed increased leukocytic (CD45+ ) infiltration (left) compared with historical control (right). Middle two panels: tumor infiltrates (CD45, left) consisted predominantly of CD3+ T lymphocytes (right). Lower two panels: the T cell population included both CD8+ (left) and CD4+ (right) lymphocytes. (G–I) Several markers of immune cell activation were also detected in the ParvOryx-treated tumor: (G) granzyme B (left) and perforin (right), (H) IFN-g (left) and IL-2 (right), and (I) CD25 (left) and CD154 (CD40L) (right). Scale bars, 50 mm.
Figure 5. Evaluation of T Cell Responses to H-1PV and Glioma Antigens by IFN-g ELISpot Assay (A and B) Cellular immune responses are shown for two patients treated with ParvOryx via (A) the intratumoral and intracerebral route (patient 2-04) or (B) the intravenous and intracerebral route (patient 5-14). PBMCs were isolated at the indicated days prior to (day 0) or after (days 10–120) treatment. After incubation with appropriate stimulants, IFN-g-producing spot-forming cells (SFCs) were counted. The test stimulants were viral or glioma peptides (Table S3) or full-length viral proteins (NS1 or empty capsids made of VP1 and VP2). Phytohemagglutinin (PHA) and cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF) peptide pools served as positive control stimulants. Negative control values (unstimulated cells) ranged from 0 to 21 SFCs per million PBMCs and were subtracted from the corresponding stimulated sample values. Means (columns) and SEMs (bars) of triplicate measurements are shown. Asterisks denote statistical significance (*p < 0.05; mean SFC _x0001_ 2 SEMs > 2 negative control).
In conclusion, the ParvOryx01 trial data confirm H-1PV safety and tolerability. They provide evidence of a lack of ectotoxicity, H-1PV ability to cross the blood-brain/tumor barrier, and favorable (progression-free) survival compared with historical controls. Finally, this trial points to H-1PV capacity for establishing an immunogenic tumor microenvironment, making H-1PV an interesting candidate for further clinical development.
Material and Methods

ParvOryx01 was an open, non-controlled, three-group, intra-group dose escalation, single-center study using a good manufacturing practice (GMP)-grade pharmaceutical formulation of H-1PV (ParvOryx). Its design is reported in Geletneky et al.24 and depicted in Figure 1. Primary objectives included ParvOryx safety and tolerability assessment, MTD determination, and viremia and H-1PV shedding investigation. Secondary objectives were proof-of-concept, PFS6, and OS6. Whenever applicable, patients were followed up for OS beyond the regular study follow-up period of 6 months by means of telephone interviews or continuing visits of the trial center.

The investigated safety and tolerability parameters were: (serious) AEs, 12-lead electrocardiograms, body temperature, blood pressure, heart rate, clinical chemistry, hematology, and clotting. Vg concentrations in blood, urine, saliva, and feces were determined by quantitative real-time PCR at screening, daily between study days 1 and 18, and at each follow-up visit. In G2, two additional blood samples were taken on each day of intravenous administration according to a preset schedule.

Fluorescence in situ hybridization (FISH), Real-time PCR, Immunofluorescence, Immunohistochemistry, detecting infectious H-1PV, H1PV DNA sequencing, vG quantification in fluid, neutralizing antibody essay, statistical planning and analysis.
THANK YOU FOR ATTENTION