Novel Antivirals for Human CMV Infection During Pregnancy

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2016 CMV Public Health and Policy Conference
Austin Texas
OUTLINE

- The placenta and CMV
- Placental and other models of congenital CMV
  - Explant models over time
  - Methods
  - Explants and CMV infection
- Potential interventions for CMV
  - siRNA inhibitors of CMV infection
  - Quinazoline inhibitors of CMV infection
- Summary
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Vertical transmission

- transplacental transmission (in utero)
- ascending infection (retrograde)
- intrapartum infection (labour, delivery, immediately after delivery, post natal, neonatal, including breastfeeding)
Congenital CMV observations

- Not all congenitally infected infants develop clinical disease
- Not all pregnant women with primary CMV infection transmit virus to their baby
- CMV placental infection precedes fetal infection by 8-12 weeks, suggesting blocking of transplacental virus movement
Normal (uninfected) placental tissue
1. Apoptosis of STB Layer
   Reduced Gas & Nutrient Transfer
   Increased CMV Dissemination

2. Reduced Cytotrophoblast Invasion
   Shallow Placentation
   Reduced Gas & Nutrient Supply to Placenta

3. Downstream Effects on Other Proteins
   Shallow Placentation
   Affects on Placental Development/Function

4. Loss of Immune Tolerance
   Rejection of Placenta
MCP-1 & TNF-α in Stillborn Infants

A mRNA

B CMV Infected

Uninfected

[Hamilton 2012]
Why use models for congenital CMV?

- Pregnancy issues
  - Ethically complex
  - Regulatory issues with experimental therapies TGA “In general pregnant women should be excluded from clinical trials” “The FDA does not run pregnancy trials”
  - Parental reluctance to participate, pregnancy testing during trials
  - Fetal tissues unavailable for study in vivo of normal pregnancies
  - Placental tissue difficult to obtain

- Virus issues
  - Human CMV species specific
  - Use of clinical strain surrogates (VR1814, TB40E, Merlin)
  - Use of low passage clinical strains difficult
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Types of models

- **Cell culture with trophoblasts/fibroblasts**
  - Continuous CTB lines (Jeg-3, TEV1, SGHPL4, htr8-neo)
  - Continuous fibroblast lines (MRC5, HFF)
  - Primary trophoblast cell lines

- **Mixed cell models**
  - Explants short term
  - Whole placentae

- **Animal models**
  - Mouse
  - Guinea pig
  - Primate
CMV upregulation of CCL2 expression temporally localises with activation of CCL2 transcriptional activators NF-κB, IRF-3, IFN-β

(a) AD169  UV-AD169  MOCK

CMV IE/E

CMV (IE/E)/DAPI

CMV (pp65)/DAPI

CMV (pp65)/DAPI

CMV (pp65)/DAPI

(b) AD169  UV-AD169

Log Viral Load (Copies/mL) vs Day

[Hamilton 2013]
Ex vivo placental explant culture

- Primary
- Explants short term
- Use to model natural infection using multicellular model
- Comparison with findings in natural infection of placenta, amniotic fluid, and single cell culture models
- Allow perturbation of infection with antivirals, novel antivirals, siRNA
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Infect Underlying Cell Monolayer with CMV

Incubate for 5 Days

Dissect Placenta and Place on Collagen Sponge Gels

Incubate Placentae with Underlying Infected Monolayer

Incubate for 5 days

Transfer Explants to Fresh Wells with No Monolayer

Incubate for a Further 7 days

Harvest Explants

DAY -5

DAY 0

DAY 5

DAY 12
CMV Productive Infection (Explants)

AD169

Merlin

IE/E  pp65  gB

IE/E  pp65  gB
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Summary of components of potential comprehensive approaches for prevention of congenital CMV through maternal and neonatal interventions

**Diagnosis**
- Targeted testing affected populations:
  - hearing loss failing SWISH,
  - stillborn,
  - SGA infants
- Bio-banking amniotic fluid from affected pregnancies

**Prevention**
- Education programs for:
  - GPs,
  - obstetricians,
  - midwives, and
  - RANZJOG trainees
- Educating pregnant women on prevention strategies through healthcare

**Monitoring**
- Register of children treated with antiviral
- Register of women treated in pregnancy with IVIG

**Therapy**
- Children with ValtGCV where symptomatic
- Pregnant women with IVIG when reported

**Health economic analyses** of congenital CMV disease and therapy costs
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siRNA Inhibition of Target Expression by Plasmid Constructs 293T cells, infection 72hr Post Plasmid Transfection

<table>
<thead>
<tr>
<th>siUL54A</th>
<th>siUL54B</th>
<th>siSc</th>
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**pUL54**

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**B**

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**IE2p86**

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siRNA Inhibition of CMV Protein Expression After Single Round of Replication (1 pfu/cell) 72hpi

72 hr Post Infection

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Relative Protein Expression (%)

[Hamilton 2014]
siRNA Inhibition of CMV Progeny Production After Single Round of Replication (1 pfu/cell) 72hpi MRC-5

Supernatant 72 hr post infection

Cells 72 hr post infection

A

B

[Hamilton 2014]
siRNA Inhibition of CMV Protein Expression After Multiple Rounds of Replication (0.001 pfu/cell) 7dpi MRC-5

Day 7

UL54b UL97a UL122b Sc No

IE1p72
IE2p86
pp65
pUL97
MCP
β-actin

Relative Protein Expression (%)
siRNA Inhibition of CMV Progeny Production During Multiple Rounds of Replication (0.001 pfu/cell)
siRNA Inhibition of CMV Dissemination After Multiple Rounds of Replication (0.001 pfu/cell) 7dpi
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Antivirals Undergoing Clinical Trials Inhibit CMV (Merlin) Replication in TEV-1 Cells (7dpi)
Antivirals Undergoing Clinical Trials Inhibit CMV (AD169) Replication in Placental Explants
Phenotypic AVS assays show virus resistant to GCV, MBV, Multi drug were susceptible to quinazoline compounds with EC50 shown

<table>
<thead>
<tr>
<th>Strain</th>
<th>UL97 genotype</th>
<th>Vi7392 Mean conc. (μM) ± SD#</th>
<th>Vi7453 Mean conc. (μM) ± SD#</th>
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<tr>
<td>T3261</td>
<td>wt</td>
<td>1.74 ± 0.51</td>
<td>2.78 ± 0.87</td>
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<td>Maribavir-resistant mutants</td>
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<td>V353A</td>
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<td>2.10 ± 0.88</td>
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<td>T409M</td>
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<td>2.48 ± 0.67</td>
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<td>Ganciclovir-resistant mutants</td>
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<td>M460V</td>
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<td>1.59 ± 0.51</td>
<td>2.47 ± 0.98</td>
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<tr>
<td>P-loop mutant (multi-drug-resistant)</td>
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<td>F342S</td>
<td>0.96 ± 0.25</td>
<td>1.80 ± 0.68</td>
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The chemical class of quinazoline compounds provides a core structure for the design of anticytomegaloviral kinase inhibitors

Novel Quinazoline (Vi7392) Inhibition of CMV in Placental Models

**TEV-1 Trophoblast Cells**

- Merlin-Infected TEV-1 Trophoblast Cells (1 pfu/cell) 7dpi

**Placental Explants**

- CMV-Infected Placental Explants (1x10^7 pfu’s) 14dpi - 2.5µM
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