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Cytomegalovirus antiviral resistance testing

A New Assay for Detection of Mutations Associated with Antiviral Resistance in CMV

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Disclosures

- None

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Cytomegalovirus (CMV) in the transplant population

- High seroprevalence, with possibility for acute and latent disease
- One of the most common complications affecting transplant recipients
- Presentation depends on a variety of factors:
  - Type of transplant (stem cell vs. solid organ)
  - Serostatus of recipient and donor (e.g., D+/R- *vs.* D+/R+)
  - Degree of immunosuppression

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Potential clinical manifestations in the transplant population

- "CMV syndrome"
  - Fever of unknown origin >48 hours
  - Malaise
  - Decreased neutrophil count
- Pneumonitis
- Hepatitis
- Gastrointestinal disease (e.g., esophagitis, colitis)
- Central nervous system disease

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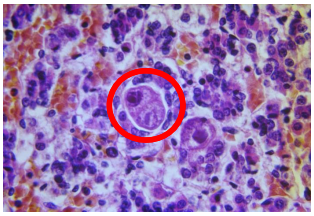
Diagnosis of CMV

- Molecular detection of CMV nucleic acid in clinical specimens
- Increasing viral load (≥0.5 log) in serially-collected plasma samples
- Histopathology of infected tissue (i.e., Owl's-eye inclusion)



https://phil.cdc.gov/Details.aspx?pid=22200

CMV disease: Presence of CMV in clinical specimen(s) accompanied by consistent clinical manifestations

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Treatment of CMV in transplant recipients

- Reduction of immunosuppression
- Antiviral therapy (ganciclovir, valganciclovir, foscarnet, cidofovir)
- Resistance to antiviral therapy may occur:
  - ~1-5% of transplant recipients
  - Results from mutation(s) in 2 primary CMV genes:
    - UL97 – can confer resistance to ganciclovir
    - UL54 – less common; can confer cross-resistance (ganciclovir/cidofovir, foscarnet)

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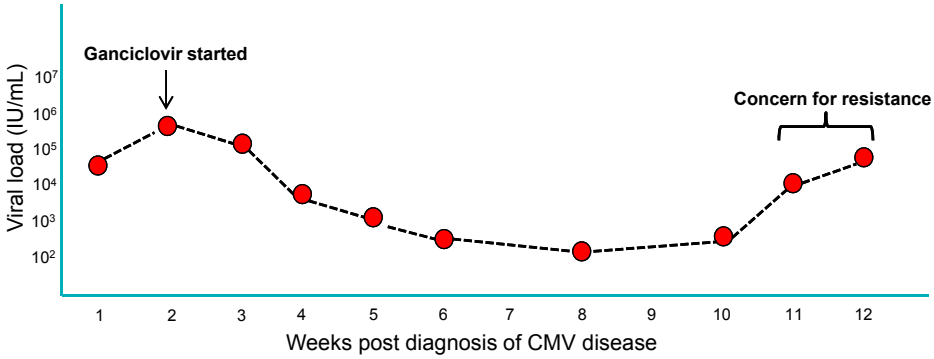
When to suspect CMV antiviral resistance?

- Rising or persistently elevated viral loads despite appropriate treatment for >2 weeks



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Options for CMV antiviral resistance testing

- Phenotypic testing
  - Requires viral culture in presence and absence of antiviral drug
  - Takes weeks to months; therefore, no longer used
- Sanger sequencing
  - Conventional sequencing of regions of DNA (i.e., UL97)
  - Can only sequence short pieces of DNA (300-900 bp)
  - Sequence quality drops after ~700 bp
  - May not be able to accurately differentiate mixed populations of sequence

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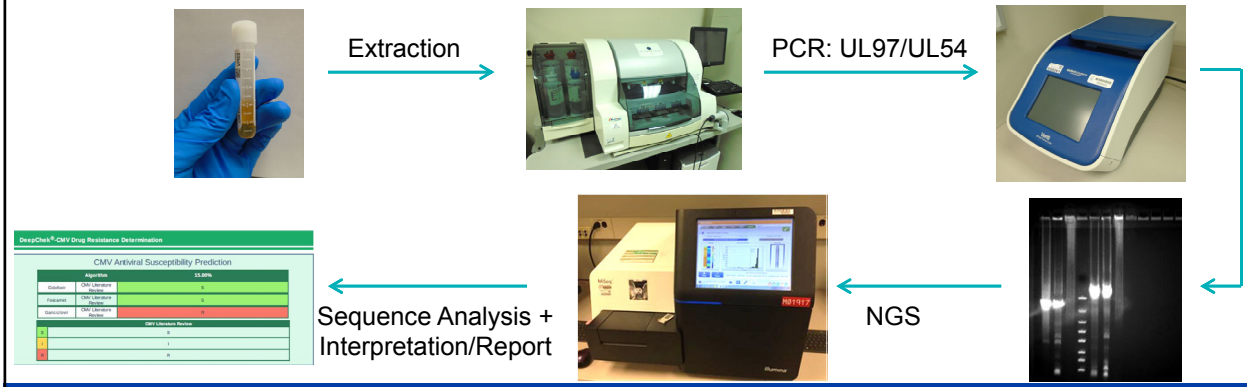
Future option for CMV antiviral resistance testing

- Next-generation sequencing (NGS) assay for detection of mutations in UL97/UL54 associated with resistance



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CMV next-generation sequencing assay

- Implemented at Mayo Clinic Laboratories on May 2, 2019
- Requires a plasma sample with a CMV viral load of ≥500 international units (IU)/mL (Sanger typically requires viral load ≥1,000 IU/mL)
- Demonstrated 97.7% (43/44) overall agreement with Sanger sequencing for resistance-level determination
- Can identify resistance-associated mutations at a prevalence threshold of ≥15% (*vs.* ≥20% by Sanger)

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Summary

- CMV is an important cause of disease in transplant patients
- Resistance to antivirals can occur and results from specific mutations in:
  - UL97 – resistance to ganciclovir
  - UL54 – resistance to multiple antivirals
- Next-generation sequencing offers a novel approach to detect mutations associated with antiviral resistance

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References

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Thank you.