

# **Biomarkers in Human Pathology and Host Response to Filovirus Infections**

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# Biomarker

**As defined by the FDA:**

***A characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes or pharmacological responses to a therapeutic intervention***



# Human Filovirus Disease

- ◆ **Incomplete picture of human pathology and events leading to severe disease, but has characteristics similar to septic shock**
- ◆ **Few opportunities to study infections**
- ◆ **Disease characteristics not identical to those of animal models, but the disease in nonhuman primates is very similar**



# Studies of Human Infections

- ◆ **Early outbreaks of filovirus disease in humans provided limited information**
- ◆ **Recent episodes have provided better insights into the disease process**

**1994 Ebola Ivory Coast virus– Tai Forest, Ivory Coast**

**1995 Ebola Zaire virus – Kikwit, DRC**

**1996-2002 Ebola Zaire virus – Gabon**

**2000-2001 Ebola Sudan virus – Gulu, Uganda**

**1998-2000 Marburg virus – Watsa, DRC**

**2004-2005 Marburg virus – Uíge, Angola**



# Biomarkers in Human Pathology

- ◆ **Filovirus**
- ◆ **Host**



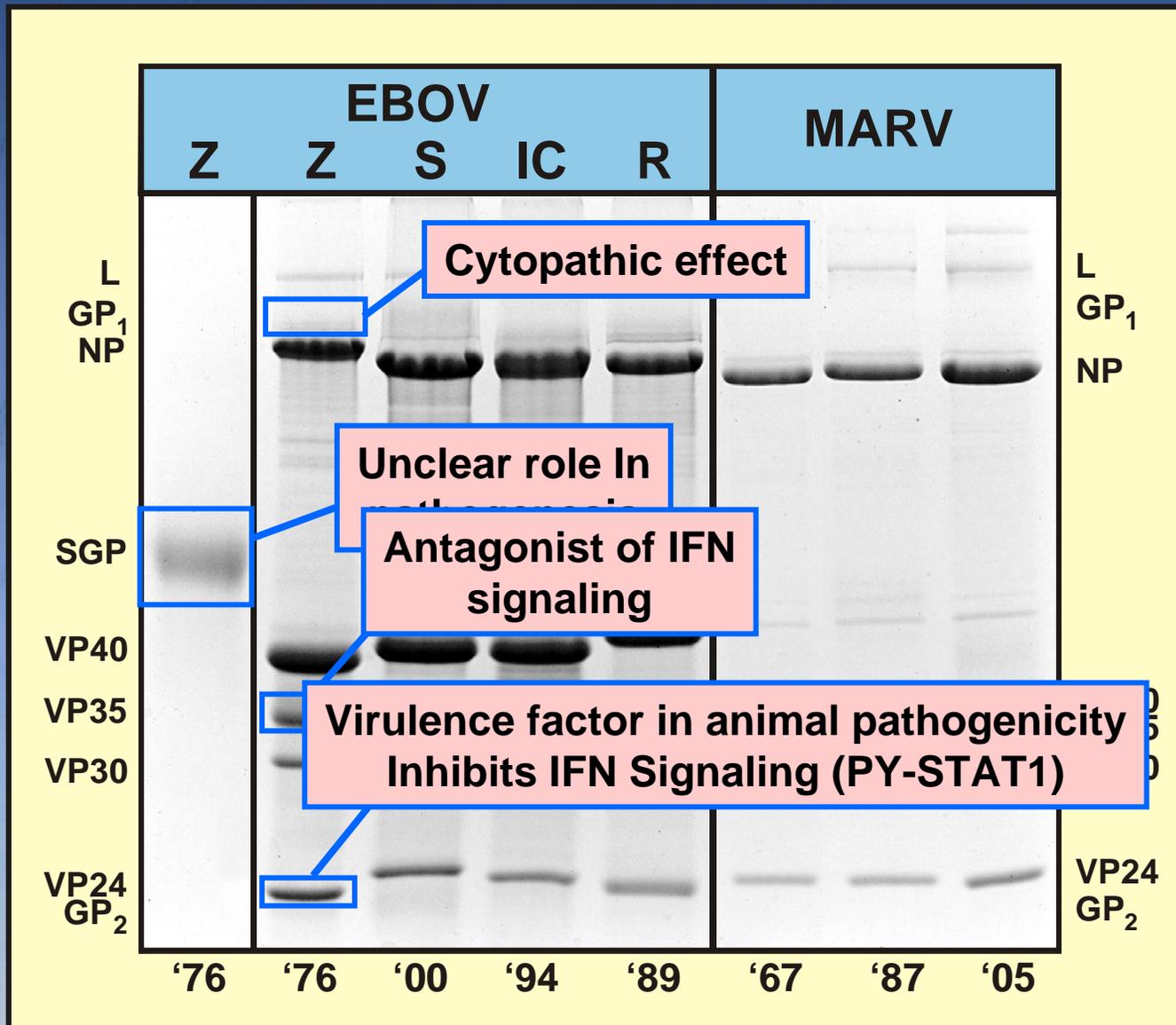
# Filovirus Biomarkers

- ◆ **Proteins**

- ◆ **RNAs**



# Filovirus Proteins



# Filovirus Protein Detection

- ◆ **IFA Assays**
- ◆ **Antigen Capture ELISA**
- ◆ **Immunohistochemistry**
- ◆ **Immunoblot Assays**
- ◆ **Flow Cytometry**



# RNA Detection – RT-PCR

- **Conventional – Agarose Electrophoresis**
- **Real-time Detection – FRET Quantitation**
  - **TaqMan**
  - **Molecular Beacons**



# Virus Load

- ◆ **Filovirus antigen or RNA quantitation can be used to determine virus loads**
- ☠ **High virus loads are associated with fatal outcomes, while lower levels are associated with milder disease**

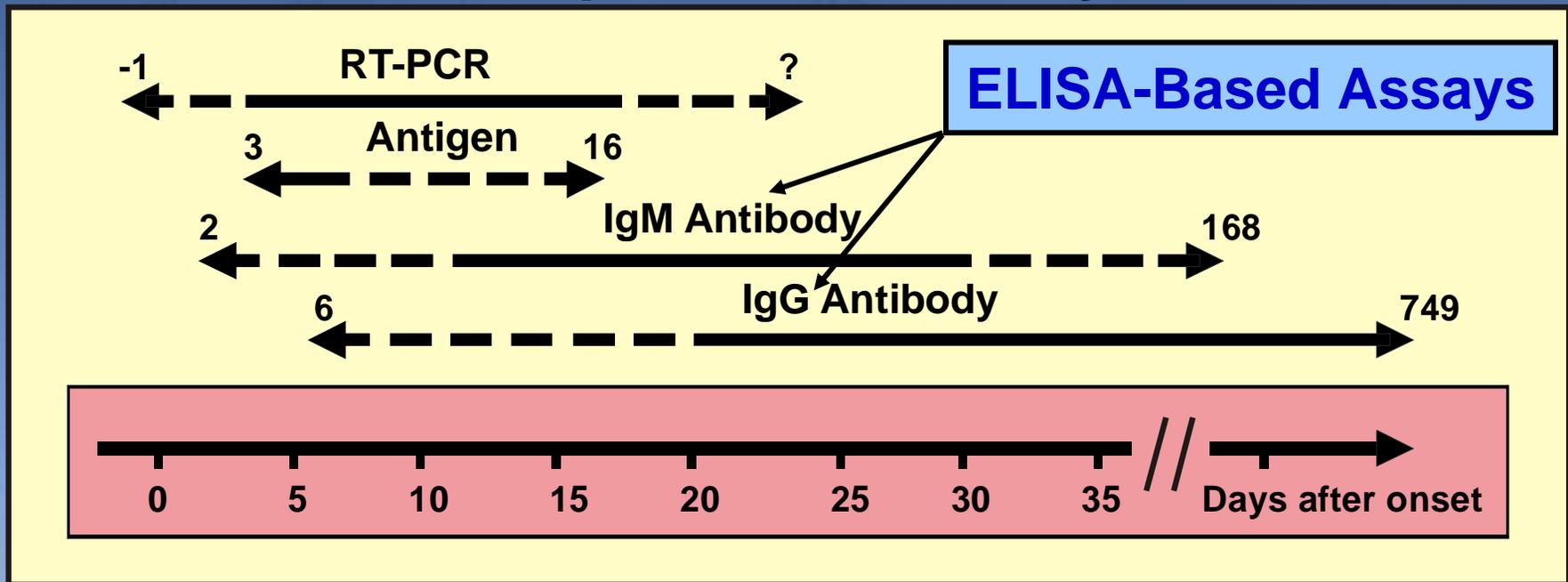


# Host Biomarkers

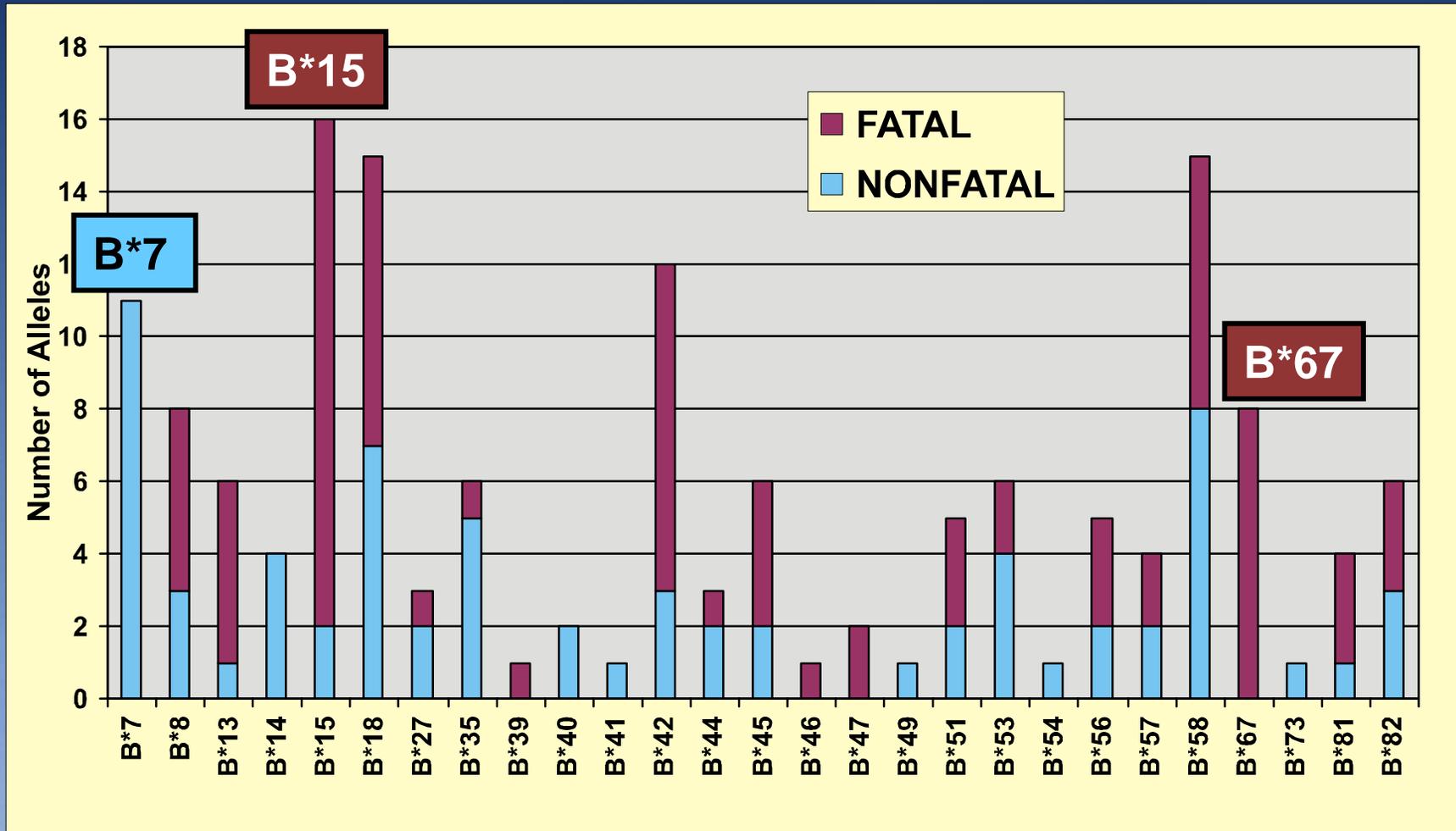


# Humoral Immune Response

- ◆ **Humoral responses are delayed**



**enhance virus entry**



- ◆ In Ebola Sudan patients, fatal and nonfatal outcomes are linked to HLA-B profile

# Cytokine Production

- ◆ **Cytokine expression is induced, but their role in stemming or enhancing the progression of disease is unclear**
- ◆ **Prolonged exposure to virus antigen may induce an unresponsive state (immune inactivation)**

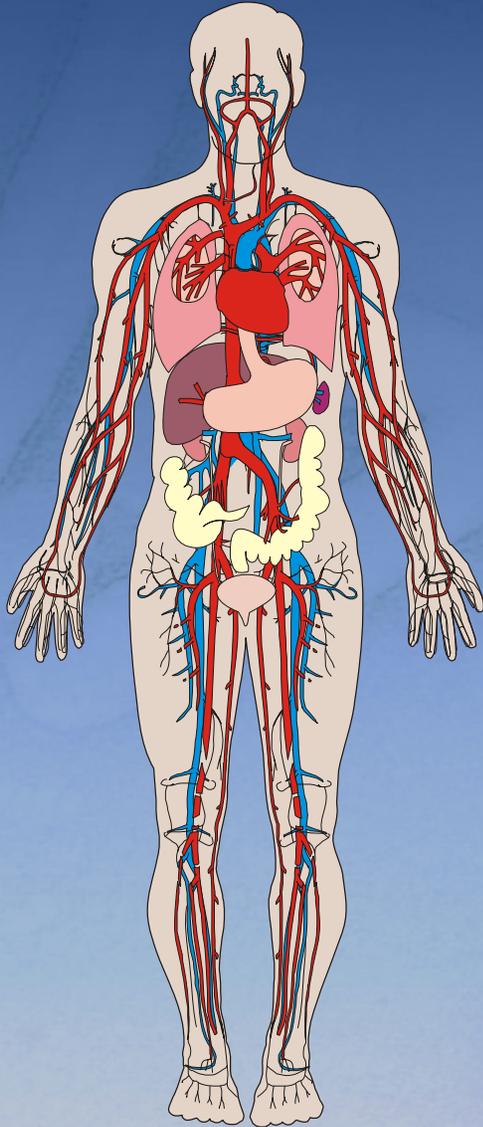


# Apoptosis

- ◆ **Leukopenia and appearance of atypical lymphocytes (pseudo-Pelger-Huet cells) likely due to unregulated apoptosis**
- ☠ **Associated with fatal outcomes in Ebola Zaire patients**



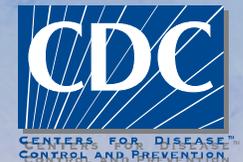
# Abnormal Blood Chemistries Associated With Fatal Disease Outcomes



- ☠ Nitric Oxide ↑
- ☠ D-Dimers ↑
- ☠ AST ↑
- ☠ Amylase ↑
- ☠ BUN ↑
- ☠ Creatinine ↑
- ☠ Albumin ↓
- ☠ Calcium ↓

# Dangerous Levels

TEST	LEVEL ASSOCIATED WITH FATAL DISEASE
Nitric Oxide	>80 $\mu$ M
D-Dimer	>100,000 ng/mL
AST	>400 u/L
BUN	>60 mg/dL
CRE	>5 mg/dL
ALP	>200 u/L
AMY	>150 u/L
ALB	<2.5 g/dL
CA++	<6 mg/dL



# Conclusions & Observations

- ◆ **There is a assortment of filovirus and host biomarkers that can be measured during human infections as well as in animal models**
- ◆ **Recent advances in studies of filovirus entry, replication, pathogenesis, and host responses should provide an impetus to the development of novel therapeutics and vaccines**
- ◆ **Relevant animal models will be key to this development**



# Uganda 2000-2001

