kidney.[38] Mice begin to lose weight and become lethargic by day 4 post-infection. As the disease progresses, mice continue to lose weight and their general appearance declines, with most animals exhibiting signs of severe disease such as ruffled fur, hunched posture and unsteady gait. By day 8 post-inoculation, mice are moribund and have lost as much as 30% of their initial body weight.

Mortality is the primary endpoint in this model, with 100% of mice succumbing to infection by day 10 post-inoculation. Disease progression can be monitored by measuring the change in weight during the course of infection. The change in weight relative to placebo-treated animals is a quantifiable, non-invasive method of measuring disease severity and correlates with systemic replication of virus in mice. The percentage weight change is useful for determining disease severity when treatment protects mice from lethal infection. Thus, the efficacy of compounds that prevent mortality but do not completely inhibit virus replication and all aspects of disease progression can be assessed in this model. To quantify the level of virus spread, animals must be sacrificed at selected time points post-infection and virus titers measured in the liver, spleen, lung, kidney and other organs. Antiviral efficacy is measured by the decreased mortality, inhibition of virus-induced weight loss and reduction in viral titers in liver, spleen, lung, kidney and other tissues.

4.3.2.2 Ectromelia Virus Mouse Model

Ectromelia virus is a laboratory pathogen of mice that has been used as a model system of OPV disease pathogenesis.[33] The pathogenesis of ectromelia virus disease closely resembles human smallpox, with distinct stages of localized replication, systemic virus spread and lesion formation; however, the time course of infection and disease progression is much shorter.[33,39] Unlike vaccinia and cowpox viruses, small amounts of virus delivered to peripheral sites can initiate a lethal infection in susceptible mice.

Ectromelia virus enters through abrasions in the skin and replicates in local lymphoid cells.[33,40] Virus can be detected in these tissues by immunofluorescence within a few hours after inoculation.[40,41] The virus multiplies in the lymphatic endothelial cells, macrophages and lymphocytes within the node over a period of 2–4 days.[33] Following this latency period, the virus spreads through the lymph and enters the bloodstream to cause a primary viremia. The virus is rapidly removed by macrophages lining the sinusoids of the liver, spleen and bone marrow.[41] Infection of the parenchymal cells of liver and lymphoid cells of spleen produces high virus titers that are released into the blood stream to cause a secondary viremia.[40] In highly susceptible animals, replication in liver and spleen produces focal necrotic lesions, acute hepatitis and multi-organ failure. In mice that are less susceptible to infection, a rash develops following the secondary viremia.[39] The rash is caused by virus replication in the perivascular cells and dermal endothelial cells and epidermis.

A lethal ectromelia virus mouse model has been established to evaluate the efficacy of antiviral drugs.[42] In susceptible mice, as little as 1 PFU of ectromelia