5. NON-CLINICAL PHARMACOLOGY AND TOXICOLOGY

5.1 Virology

Antiviral studies have not been conducted with valganciclovir *in vitro* as the effective antiviral properties are those of ganciclovir itself. The historical findings related to ganciclovir up until 1994, and an overview of more recent findings to 1999 are documented in the Non-Clinical Virology Summary of Ganciclovir in support of valganciclovir.

Ganciclovir is specifically phosphorylated to its monophosphate by the HCMV-encoded kinase, UL97. Cellular enzymes then complete production of the active metabolite, ganciclovir tri-phosphate (GCV-TP). The primary mechanism of the drug's action is through inhibition of HCMV DNA polymerase (UL54) by GCV-TP. Antiviral activity is prolonged after depletion of extracellular ganciclovir because of slow intracellular catabolism resulting in a long intracellular half-life of the tri-phosphate (6-24 hours). Recent biochemical investigations with recombinant vaccinia virus containing the viral protein kinase UL97 support the role of this enzyme in the selective initiation of ganciclovir activation in virus infected cells.

Ganciclovir shows potent antiviral activity against a range of different laboratory strains and clinical isolates of HCMV, with activity (IC_{50} values) in the range of 0.08-14 μM (0.02-3.58 μg/mL) and shows potent antiviral activity against all known human herpes viruses. Furthermore, ganciclovir shows additive properties of antiviral efficacy in *in vitro* studies with foscarnet (PFA), adefovir (ADV) and recombinant human interferon (rHuIFN)-α and additive or synergistic activity with cidofovir (CDV). In addition, synergy between ganciclovir and rHuIFN-β has been demonstrated. Ganciclovir does not affect anti-HIV antiviral activity of azido deoxythymidine (AZT), although antagonism has been reported under conditions of excess ganciclovir.

In vivo, ganciclovir treatment of murine CMV (MCMV)-infected mice is found to increase survival rates significantly. Antiviral activity is also observed in chemically or genetically immunosuppressed mice, although the extent of the effect is less than that observed in normal mice. However, antiviral activity has been demonstrated in guinea pig CMV (GPCMV)-infected guinea pigs, despite relatively poor activity against this virus *in vitro*.

Several amino acid residues in both UL97 and UL54 have been implicated in resistance to ganciclovir, and many have been confirmed as contributing to resistance in studies of recombinant viruses in which a wild type genotype is substituted with the single mutation under examination. The mutations occur in clusters in conserved regions of the genes. Certain regions are critical for enzyme activity and the incorporation of mutations can reduce the virus' ability to grow and modulate its properties. Resistance to ganciclovir is more likely to arise initially through mutation of the UL97 viral gene. Recent data

suggest that IC_{50} levels in the range 6-12 μM may indicate a low level resistance associated with a mutation in the UL97 gene. As ganclovir is the only currently licensed anti-CMV drug that requires this enzyme, mutations in UL97 are unlikely to give rise to cross-resistance with other approved drugs within the indication.

5.2 Non-Clinical Pharmacokinetics and Drug Metabolism

A cross-species comparison of the pharmacokinetics and metabolism of valganciclovir has been carried out to determine the suitability of this compound as a pro-drug for delivering ganciclovir with a higher oral bioavailability. The species that were studied (the mouse, rat, dog and cynomolgus monkey), were chosen on the basis of previous knowledge of the fate of ganciclovir so that comparisons could be readily made. There is a large database of information on ganciclovir in man with respect to both efficacy and safety information. Therefore, the focus of this non-clinical development has been to determine the pharmacokinetic characteristics of this pro-drug and its ability to deliver ganciclovir in a reliable and predictable manner such that the non-clinical studies can be considered to be a good indicator to man.

All of the studies for the development of valganciclovir have used a similar analytical method, HPLC-UV for valganciclovir and HPLC-fluorescence for ganciclovir. Valganciclovir hydrolyses relatively slowly in plasma despite its rapid hydrolysis by both intestine and liver esterases to ganciclovir. Plasma was obtained

These storage periods are suitable for all the species investigated during this development program, mouse, rat, dog and man. Since ganciclovir is stable its stability was not a concern.

The bioavailability of oral ganciclovir is poor in man (~6%), rat and cynomolgus monkey (7-10%), whereas valganciclovir is rapidly absorbed and extensively hydrolyzed delivering good bioavailability of ganciclovir in all species tested, 50% in cynomolgus monkeys, 56% in rats, 60% in man and 100% in dogs and mice. Since ganciclovir is the only metabolite of valganciclovir and ganciclovir is excreted almost wholly unchanged via the kidney, these figures also represent the absorption of valganciclovir.

The distribution pattern of intravenous doses of radiolabelled material following administration to rats is essentially the same for valganciclovir as for ganciclovir. The dose is distributed to all well-perfused organs and tissues in agreement with valganciclovir's measured distribution volume, 0.3 L/kg in dogs and 0.45 L/kg in cynomolgus monkeys, which equates to slightly more than extra-cellular fluid. The dose is rapidly excreted, predominantly via the kidney where this organ is exposed to 5 fold higher concentrations than any

other tissue. Protein binding for ganciclovir is low, <2% in all species, including man, and is the same across concentrations from 2-200 μM. Although protein binding has not been measured for valganciclovir, it is unlikely to be more extensively bound because the valyl ester is less lipophilic.

The pharmacokinetics of valganciclovir are linear with respect to dose and independent of time up to doses of ~400 mg/kg with no evidence of saturable absorption. In repeat dosing studies there is no accumulation of valganciclovir or ganciclovir and no difference in the pharmacokinetics of either compound in male or female animals. The systemic exposure of valganciclovir is low and transient in all species tested and none could be detected in hepatic portal or systemic cynomolgus monkey plasma. The AUC relative to that of ganciclovir is 1-2% in dogs, 2-4% in mice, and 4-8% in rats. This low figure is due to its rapid hydrolysis to ganciclovir with resultant short half-lives of 5 minutes in mice, 13 minutes in rats and 30 minutes in the dog and cynomolgus monkey.

The toxicokinetic data for ganciclovir have been compared following oral and i.v. doses of valganciclovir and ganciclovir. Comparable plasma exposures show the same adverse event profile with no new findings with valganciclovir. The safety aspects of valganciclovir were further studied by avoiding first pass metabolism, and increasing the AUC of valganciclovir relative to ganciclovir to 80% by administering an i.v. dose of valganciclovir to mice for two weeks. The high plasma exposure of valganciclovir in this study, where the AUC of valganciclovir was ten times the maximum anticipated in man, showed no adverse effects other than those attributable to ganciclovir. This suggests a safety margin for valganciclovir of at least 10 fold.

5.3 Toxicology and Safety Pharmacology

The toxicology and safety pharmacology program for valganciclovir conducted in the same species as used for ganciclovir demonstrate that its toxicological profile is directly comparable and essentially similar to the well-documented toxicity profile of ganciclovir. Since the systemic exposure of ganciclovir was the main determining factor in inducing toxicity, the chronic toxicity, reprotoxicity, and carcinogenicity studies undertaken for ganciclovir were not repeated with valganciclovir as similar results were expected.

Safety pharmacology studies were performed on the nervous, gastrointestinal, cardiovascular and renal systems, and on gross behavior. Except for an increase in urine volume and an imbalance in urine electrolytes in rats administered a dose of 150 mg/kg/day and higher, no other adverse results were recorded. Consistent with this result was an increase in urine volume in female rats administered 200 mg/kg/day ($P < 00.1$ compared to controls) for 13 weeks where no renal pathological changes were identified.

Acute oral doses of valganciclovir to mice (up to 2000 mg/kg/day) and dogs (up to 1000 mg/kg/day) did not result in any effects in mice at the time of dosing, but did induce vomiting in dogs within 3 hours of administration of the high dose. In the dog, no histopathological changes were found, but during the observation period, white blood cells and platelets declined in males administered 500 and 1000 mg/kg and in females administered 500 mg/kg. One mouse was found dead in the 2000 mg/kg/day/dose group on day 2, for which a cause of death was not established.

The duration of multiple dose toxicology studies were 3 months for the rat and dog and 6 months for the mouse. The toxicity of ganciclovir has been characterized in previous studies in mice, rats and dogs as testicular atrophy. More variably reported were ovarian atrophy, leukopenia, atrophy of the thymus, spleen, lymph nodes and particularly bone marrow, and anemia and thrombocytopenia. Apart from the testicular atrophy and advanced renal and intestinal changes, all findings were reversible upon withdrawing treatment. No additional toxicity features were seen other than those expected for the ganciclovir exposures achieved. The same target organs were affected with both valganciclovir and ganciclovir namely: the reproductive system followed by the hematopoietic, renal, intestinal and adnexal systems, the degree of effect being related to the exposure of ganciclovir achieved. In the clinic, these adverse results correspond to symptoms of anemia, leukopenia, thromboytopenia, nephrotoxicity and intestinal disturbances.

Ganciclovir and valganciclovir were mutagenic in mouse lymphoma cells and clastogenic in mammalian cells. Such results are consistent with the positive carcinogenicity study in mice with ganciclovir where multiple tumors were induced in organs having human counterparts (intestine, female reproductive system, skin, and liver). It is assumed therefore that valganciclovir, like ganciclovir, is a possible carcinogen and carries the same prescribing information warning.

Toxicity was induced by ganciclovir exposures equal to or lower than the therapeutic human exposure. However, the therapeutic dose of valganciclovir has been selected to give a ganciclovir AUC comparable to that achieved with current intravenous ganciclovir where the safety profile is well known and manageable in clinical practice. The highest exposure achieved for valganciclovir following i.v. doses to mice was 10 times greater than the maximum clinical dose of 900 mg/b.i.d. At this dose, no toxicological findings were recorded other than those expected from exposure to ganciclovir.

5.4 Conclusions of the Pre-clinical Program

The antiviral mechanism of action for ganciclovir on the herpes viruses (mainly CMV), has been well documented in the past. Data from recent studies with a variety of recombinant viruses, both sensitive and resistant to ganciclovir, are consistent with this mechanism.

The pharmacokinetic and metabolic studies have shown that valganciclovir is a prodrug of ganciclovir which is rapidly absorbed and efficiently hydrolysed in all non-clinical species investigated. The bioavailability of ganciclovir from an oral dose of valganciclovir is increased over that of ganciclovir by almost a factor of 10 in rats and cynomolgus monkeys and is 100% in mice and dogs. Ganciclovir is the only metabolite of valganciclovir and the tissue distribution pattern following i.v. doses of either compound is the same, supporting the premise that valganciclovir is an alternative and more effective means of delivering ganciclovir.

Non-clinical safety studies demonstrated a toxicological profile that is essentially the same as the well-documented toxicity profile of ganciclovir with no additional findings with valganciclovir. The main target organs for both compounds were the reproductive, hematopoietic, renal, intestinal, and adnexal systems.

At the highest exposure achieved for valganciclovir following i.v. doses to mice (10 times greater than the maximum clinical dose of 900 mg b.i.d.), no toxicological findings were recorded other than those expected from exposure to ganciclovir.

Toxicity is induced by ganciclovir exposures equal to or lower than the therapeutic human exposure. However, the therapeutic dose of valganciclovir has been selected to give a ganciclovir AUC comparable to that achieved with current intravenous ganciclovir where the safety profile is well known and manageable in clinical practice.

6. HUMAN PHARMACOKINETICS AND BIOAVAILABILITY

Since valganciclovir is a pro-drug whose main function is to deliver ganciclovir, it is the pharmacokinetics of ganciclovir which are more pertinent to the efficacy and safety of the product. For ease of review a summary of the pharmacokinetics of oral and i.v. ganciclovir is provided followed by the information for valganciclovir.

6.1 Human Pharmacokinetics and Bioavailability Summary for Ganciclovir

This application includes a report reviewing the current state of knowledge regarding the pharmacokinetics of oral and i.v. ganciclovir. This document is intended as supportive information to the human pharmacokinetics and biopharmaceutics summary for valganciclovir.

The data reviewed originates from internal studies used in support of previous submissions for i.v. and oral ganciclovir and from data available as published literature.