# Preclinical efficacy and safety of AIC468, a first-in-class antiviral antisense oligonucleotide for the treatment of BKV infection in kidney transplant recipients

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## Abstract

**Background**: Reactivation of BK polyoma virus (BKV) represents a major clinical problem. In patients undergoing kidney transplantation, this can lead to BKV-associated nephropathy causing chronic kidney failure or graft loss. Currently, there is no antiviral treatment to combat BKV reactivation, other than the tapering of immunosuppressive regimens in patients, an approach that significantly enhances the risk of graft rejection. AIC468 is a potent direct-acting antiviral antisense oligonucleotide (ASO) in development for treatment of BKV infection in kidney transplant recipients.

**Methods:** The antiviral efficacy of AIC468 was evaluated in a series of *in vitro* experiments using stably transformed mouse cells and BKV-infected primary human kidney cells, whereby different methods of ASO delivery were employed. In addition, AIC468 was tested in several preclinical safety and PK studies to support the use of this novel BKV-specific ASO as a promising option for BKV treatment in renal transplant patients.

**Results**: Studies in mouse cells transformed with the BKV early coding region showed that AIC468 effectively inhibits correct splicing of mRNA coding for the BKV master regulator large-T-antigen, confirming the splice-modulating mechanism of AIC468. Importantly, this mechanism of action translates into a potent antiviral activity shown in BKV-infected primary human kidney epithelial cells. Furthermore, ASO kidney concentrations obtained in minipigs revealed that a potential efficacious tissue concentration, extrapolated from *in vitro* efficacy experiments, could be achieved *in vivo*. Finally, we demonstrate that AIC468 was safe and well-tolerated in preclinical pharmacokinetic and safety studies, supporting further clinical development.

## Schematic representation of the anti-BKV ASO AIC468 and its targeting strategy

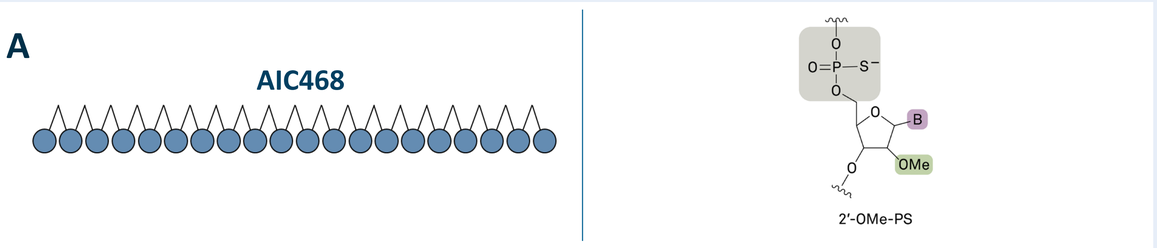
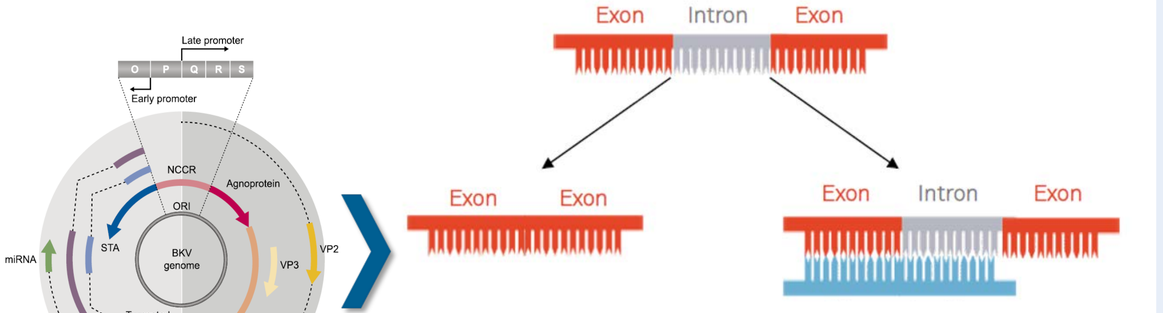



**Figure 1. AIC468 is a fully modified 2nd generation ASO targeting the BKV early coding region pre-mRNA (A) Left panel:** Schematic drawing of AIC468, a BKV specific 20mer phosphorothioate oligonucleotide. **Right panel:** Chemical modifications to the backbone and sugar moiety of AIC468 that characterize AIC468 as a 2nd generation ASO. **(B) Left panel:** Schematic drawing of BKV gene expression. The BKV genome contains three regions: a non-coding region, containing the early and late promoters, transcription sites and the origin of replication; an early region encoding the T antigens and a late region encoding the viral structural proteins. Individual proteins are expressed from a variably-spliced early or late pre-mRNA transcript. **Right panel:** Proposed AIC468 targeting strategy. AIC468 modulates the correct splicing of the early coding pre-mRNA by binding to a splice donor site, thereby preventing the expression of the BKV master regulator large T antigen (T-Ag).

## *In vitro* modulation of T-Ag splicing upon carrier mediated (transfection) and carrier free (gymnotic) ASO delivery/uptake



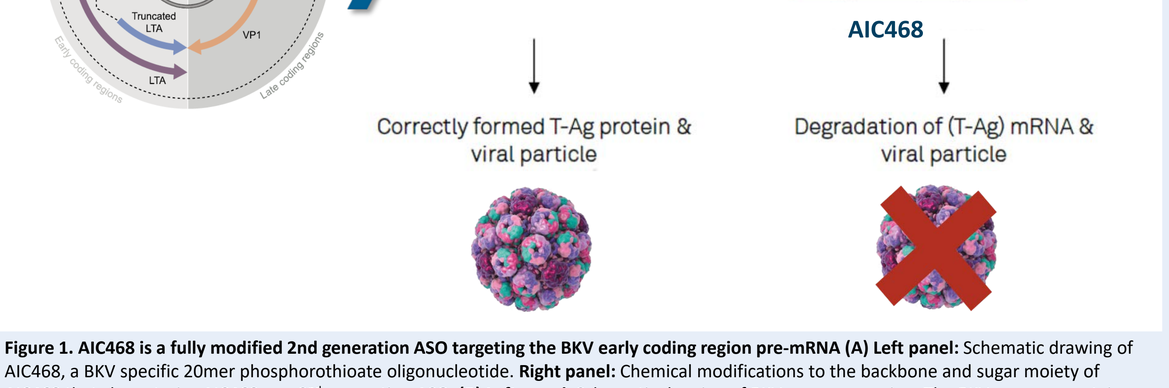




**Figure 2. AIC468 modifies T-Ag splicing in stably transformed mouse fibroblast cells (pRPC) (A)** Alternative splicing of the BKV early coding region pre-mRNA expressed in pRPC cells yields 2 main splice products encoding T-Ag mRNA or t-Ag mRNA, respectively. The AIC468 target site covering the T-Ag splice-donor sequence is indicated. **(B)** Concentration-dependent, sequence specific binding of AIC468 to the BKV early coding region pre-mRNA results in blocking of the dominant T-Ag splice site and thereby triggers alternative splicing of the t-Ag mRNA as well as aberrant splicing. Note: administration of a scrambled control ASO to pRPc in a dose-dependent fashion does not impact early coding region splicing. The specific splice-modulating effect of AIC468 is evident regardless of whether the ASO was delivered by transfection (upper panel) or carrier-free (gymnotic, lower panel). **(C)** Representative dose response curves created from the relative expression of T-Ag mRNA (measured by qPCR) in pRPc cells 24h after carrier mediated or carrier free delivery of increasing concentrations of AIC468. $EC_{50}$ values as well as the respective intracellular concentrations at $EC_{50}$ are depicted.

## Antiviral activity

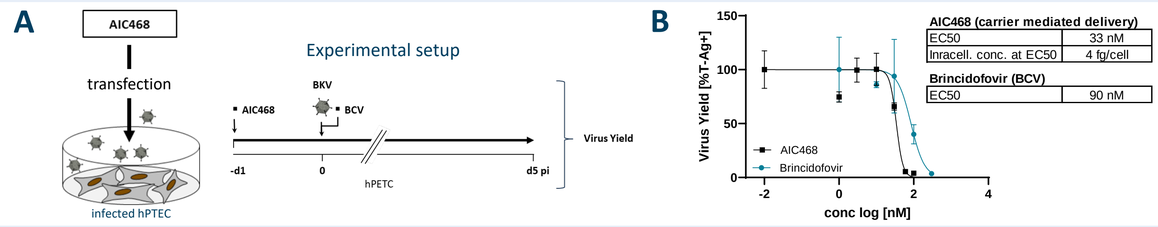



**Figure 3. AIC468 demonstrates a potent antiviral activity in BKV infected primary human kidney tubular epithelial cells (hPTEC) (A)** Schematic drawing of the antiviral assay. Cultured hPTECs were transfected on Day -1 with AIC468 at increasing concentrations in the presence of the transfection agent lipofectamine. On Day 0, cells were infected with BKV (Gardner strain) by exposure to $2.3\times10^7$ DNA copies/mL for 2 h. On Day 5, PTEC cell viability and intracellular ASO concentrations were evaluated, as well as the release of infectious progeny viral particles as a measure of antiviral efficacy ($EC_{50}$). The small molecule inhibitor brincidofovir (BCV) was used as a control compound for BKV inhibition*. **(B)** Dose response curves created from the relative numbers of BKV infected cells obtained 3 days after infection of hPTECs with 3x diluted cell culture lysates derived from BKV-infected and drug treated cells (virus yield release assay). AIC468 black; BCV blue. The respective $EC_{50}$ values as well as the corresponding intracellular concentrations of AIC468 are depicted.

*Rinaldo, C.H., et al., *1-O-hexadecyloxypropyl cidofovir (CMX001) effectively inhibits polyomavirus BK replication in primary human renal tubular epithelial cells.* Antimicrob Agents Chemother, 2010. **54**(11): p. 4714-22.

## Prediction of an efficacious kidney concentration

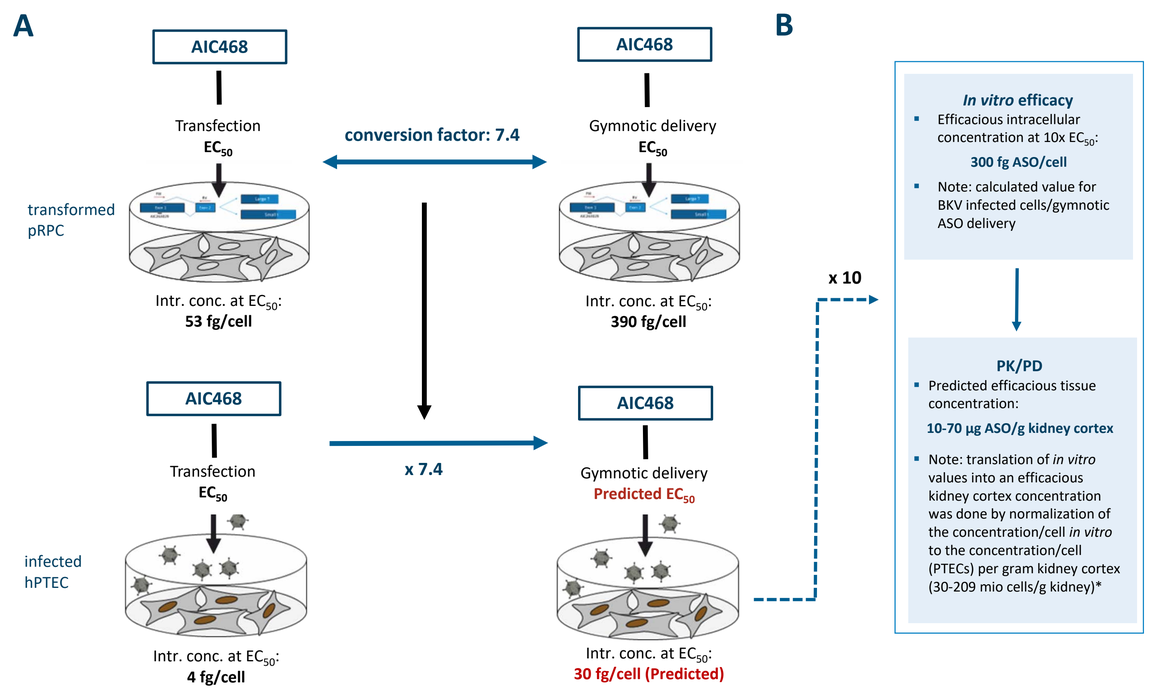



**Figure 4. Calculation of an efficacious kidney concentration on the basis of *in vitro* efficacy data. (A)** Strategy to calculate an $EC_{50}$ value for infected hPTECs upon carrier free delivery of AIC468. Primary human PTECs are susceptible to BKV infection but are not suitable for gymnotic uptake of ASOs. Accordingly, stably transformed pRPC cells were used to determine a conversion factor suitable for converting intracellular concentrations of AIC468 obtained after transfection to concentrations obtained by carrier-free delivery. Assuming that the same factor applies to hPTECs, a potential effective intracellular concentration at $EC_{50}$ was calculated for AIC468 delivered carrier-free to BKV-infected primary epithelial cells. **(B)** To achieve complete inhibition of BKV replication, a tenfold concentration of the *in vitro* $EC_{50}$ was used to calculate a potential effective AIC468 kidney concentration

*Scotcher D.; et al. A. Key to Opening Kidney for In Vitro-In Vivo Extrapolation Entrance in Health and Disease: Part I: In Vitro Systems and Physiological Data. AAPS J. 2016 Sep;18(5):1067-1081.

## AIC468 kidney accumulation and pharmacokinetics

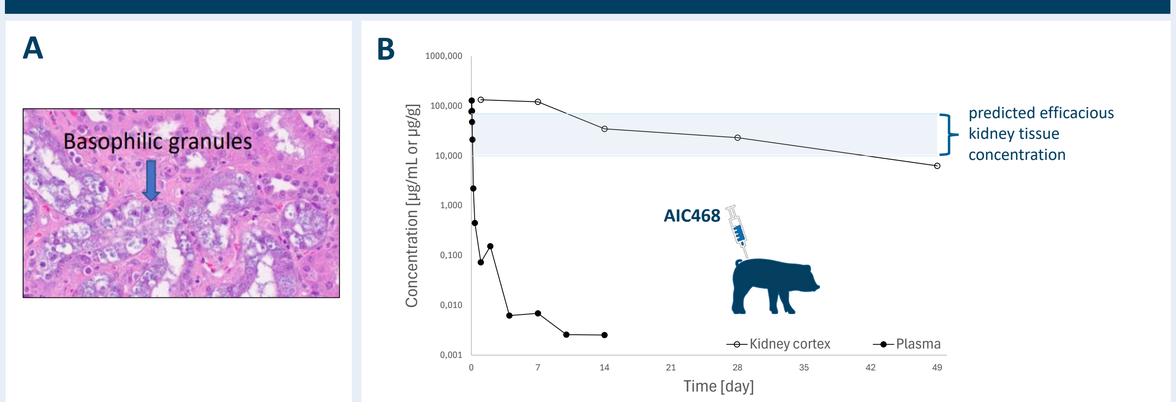



**Figure 5. AIC468 accumulates in minipig kidney tubular epithelial cells at concentrations covering the predicted efficacious concentration. (A)** Hematoxilin & Eosin staining of minipig kidneys dosed with AIC468. Bilateral cytoplasmic basophilic granules in kidney tubular epithelium of minipigs are indicated. Those granules are considered to represent accumulation of test item* and have been observed with AIC468 across all dose levels tested. **(B)** Pharmacokinetics of AIC468 in minipigs: Plasma and kidney samples were analyzed over 7 weeks following a single intravenous 1-hour infusion of 6 mg/kg AIC468 to Göttingen minipigs. The efficacious concentration range in human kidney tissue (10-70 µg ASO/g kidney cortex) was estimated based on *in vitro* $EC_{50}$ cell culture data (intracellular concentration) with normalization to the number of PTECs per gram kidney cortex. PK data revealed that efficacious concentrations in minipig kidneys can be reached with low doses of AIC468 and support a weekly or less dosing regimen.

*Braendli-Baiocco, A.; et al. The minipig is a suitable non-rodent model in the safety assessment of single stranded oligonucleotides.Toxicol Sci. 2017 May 1;175(1):112-128.

## Summary of preclinical pharmacokinetic and safety studies with AIC468

| Study | Objective | Overall summary |
|---|---|---|
| Single dose GLP cardiovascular and respiratory study in minipigs | Investigate potential effects on blood pressure, heart rate, ECG, body temperature, and respiratory rate | ▪ AIC468 was safe and well tolerated in safety pharmacological and toxicological studies by the subcutaneous and intravenous route in rodent and non rodent species.<br>▪ AIC468 has shown a favorable pharmacokinetic profile with rapid distribution from the systemic circulation into tissues.<br>▪ The target organ, the kidney, showed highest exposure with estimated half-lives of about 14 to 23 days. |
| Non GLP single and multiple dose studies in mice | Investigate plasma PK and tissue distribution after single and multiple dosing as well as initial tolerability and safety | |
| Non GLP single and multiple dose studies in minipigs | Investigate plasma PK and tissue distribution after single and multiple dosing as well as initial tolerability and safety | |
| GLP toxicity study with a subsequent recovery period in minipigs | Define a NOAEL for setting up a safe starting dose for the first human dosing | |

## Conclusions and outlook

- AIC468 is a novel, first in class, splice-modulating, direct acting antiviral antisense oligonucleotide with potent anti-BKV activity and a favorable PK and toxicity profile
- Efficacious concentrations can be covered in the target organ (kidney) in animals
- Available preclinical pharmacokinetic and safety studies support the further clinical development of AIC468 for the treatment of BKV infection in kidney transplant recipients
- A phase 1 single- and multiple-ascending dose trial in healthy volunteers is scheduled for Q3 2024

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