Preclinical Characterization of a Novel Anti-Viral Antisense Oligonucleotide (AIC468) in Clinical Development for Treatment of BKV Infections in Kidney Transplant Recipients

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Abstract

Purpose: Reactivation of polyoma BK virus (BKV) represents a major clinical problem. Especially in patients undergoing kidney transplantation, BKV can lead to BKV-associated nephropathy causing chronic kidney failure or graft loss. Currently, no approved antiviral treatment strategy exists to combat BKV reactivation, except of tapering immunosuppressive regimens in patients, an approach that greatly enhances the risk of graft rejection. AIC468 is a direct-acting antiviral antisense oligonucleotide (ASO) which inhibits the correct splicing of the mRNA coding for the BKV master regulator large T-antigen. Currently it is in development for treatment of BKV infection in kidney transplant recipients.

Methods: The drug was tested in safety pharmacological, pharmacokinetic, and toxicological studies by the subcutaneous and intravenous route in rodent and non-rodent species. Single and multiple dose studies up to 28 days followed by a subsequent treatment free recovery period up to 10 weeks were conducted to characterize *in vivo* safety and pharmacokinetics. Calculations on a potential efficacious human tissue concentration were performed in comparison to the potent antiviral efficacy of AIC468 seen *in vitro*.

Results: Here we present data on AIC468 from pivotal preclinical safety studies which support the use of this novel BKV-specific ASO as a promising option for BKV treatment in kidney transplanted patients. In a single dose study in minipigs, AIC468 exhibited no relevant effects on respiratory function, ECG parameters, body temperature and blood pressure when dosed up to the highest dose tested. *In vitro* and *in vivo* studies have shown a favorable pharmacokinetic profile with rapid distribution from the systemic circulation into tissues. The target organ, the kidney, showed highest exposure with estimated half-lives of about 14 to 23 days. A 4-week GLP toxicity study in minipigs revealed no AIC468-related effects on clinical observation, body weight, food consumption, ophthalmology, electrocardiology, or neurobehavioral evaluation and urinalysis after five intravenous or subcutaneous injections and on hematologyparameters after subcutaneous injections. A no observed adverse effect level (NOAEL) was defined and a safe starting dose for phase 1 was established. Importantly, the observed kidney concentrations in minipigs demonstrated that predicted efficacious tissue concentrations could be safely achieved i*n vivo*.

Mode of action

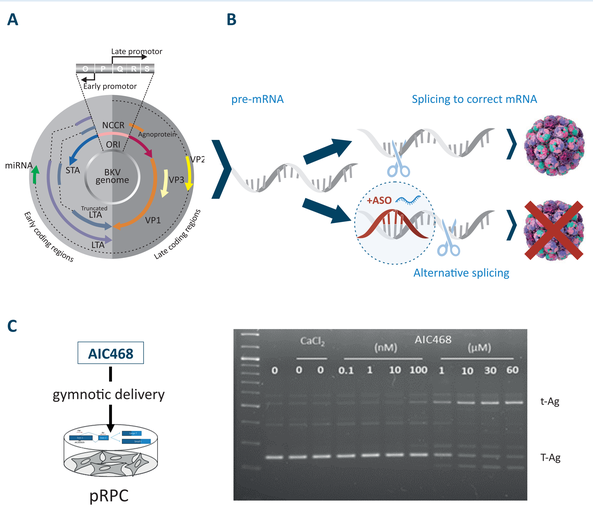
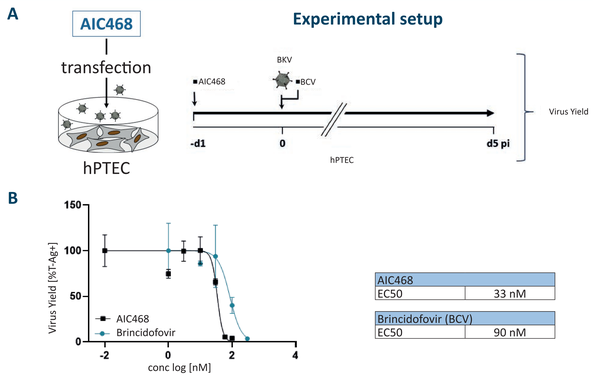


Figure 1. AIC468 is a fully modified 2nd generation ASO targeting the BKV early coding region pre-mRNA and thereby selectively inhibiting the expression of the essential viral protein large T-antigen. (A) Schematic drawing of BKV gene expression. The BKV genome contains three regions: a non-coding region, 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. **(B) 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). **(C) Splice modulating activity of AIC468 in stably transformed pRPC cells.** 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.

Antiviral activity *in vitro*



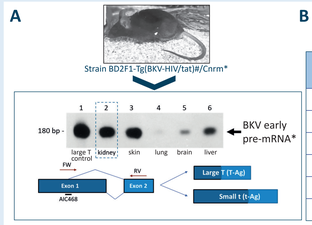
AIC468	
EC50	33 nM

Brincidofovir (BCV)	
EC50	90 nM

Figure 2. Potent antiviral activity of AIC468 shown *in vitro*. (A) Schematic drawing of the antiviral assay. Cultured human PTECs were transfected on Day -1 with AIC468 at increasing concentrations. On day 0, cells were infected with BKV (Gardner strain) by exposure to approximately 2.3×10^7 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 (EC50). The small molecule inhibitor brincidofovir (BCV) was used as a control compound for BKV inhibition*. **(B) Dose response curves created from virus yield release assay.** AIC468 black; BCV blue. The respective EC50 values of both drugs 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.

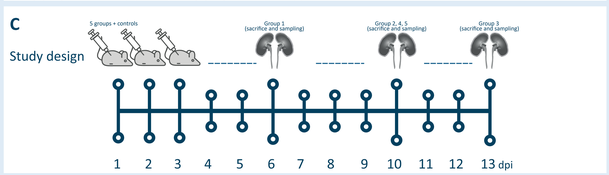
Splice modulating activity *in vivo*



B

Group	Dose (mg/kg)	Animals		Sacrifice (d)
		verum	saline	
1	3x100	5	3	6
2	3x100	5	3	10
3	3x100	5	3	13
4	3x30	5		10
5	3x200	5		10

*Corallini, A., et al., Cancer Res. 1993. 53(22): 5569-75 | Altavilla, G., et al., Am J Pathol. 1999. 154(4): 1231-44



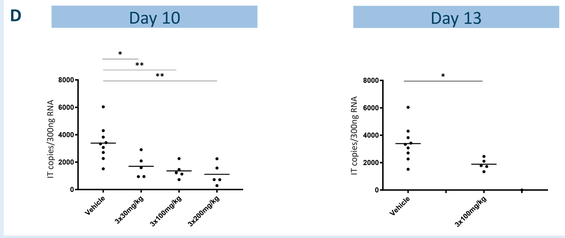


Figure 3. AIC468 significantly reduces T-Ag mRNA expression *in vivo* in a transgenic mouse model (BKV-tat mouse). (A) Depiction of transgenic mice carrying the complete BKV early coding region. The transgene is stably maintained in all organs and BKV early pre-mRNA (giving rise to T-Ag and t-AG mRNA via alternative splicing) is expressed in all tissues of the transgenic mice including kidneys. **(B+C) Study design:** Transgenic mice injected subcutaneously at 3 consecutive days with different doses of AIC468 were sacrificed on days 6, 10 and 13 and renal BKV T-Ag mRNA copies were determined by quantitative PCR. **(D) Results**: Mice sacrificed on day 6 showed no significant effect when treated with 3x100 mg/kg AIC468. In contrast, T-Ag mRNA expression was significantly reduced in all mice sacrificed on day 10 or 13 following treatment with either increasing doses of AIC468 (3x30, 3x100, 3x200 mg/kg) on day 10 or 3x100 mg/kg AIC468 on day 13.

Safety pharmacology

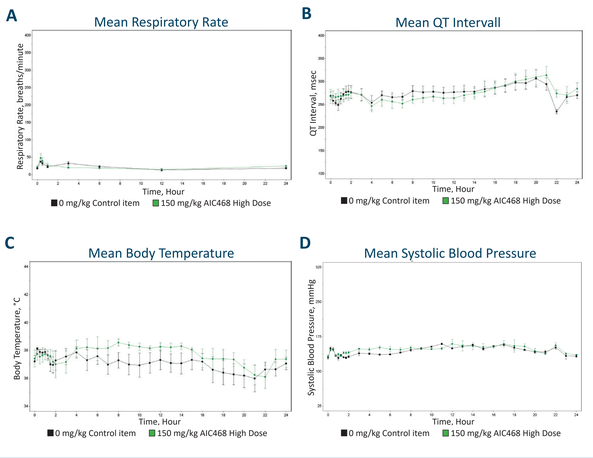


Figure 4. A single administration of AIC468 by a 5-minute intravenous infusion to telemetered Göttingen minipigs was not associated with any relevant changes in hemodynamic and electrocardiographic parameters, on body temperature and on the respiratory rate. (A) No test item-related effects on the **respiratory rate** following administration of AIC468 up to the highest dose tested (150 mg/kg). **(B)** No relevant test item-related effects on **QT interval** following administration of AIC468 up to the highest dose tested (150 mg/kg). **(C)** No relevant test item-related effects on **body temperature** following administration of AIC468 up to the highest dose tested (150 mg/kg). **(D)** No relevant test item-related effects on **blood pressure** following administration of AIC468 up to the highest dose tested (150 mg/kg). AIC468 green; Control black.
All animals dosed with AIC468 were systemically exposed to AIC468.

Toxicology

Study	Objective	Overall summary
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	• AIC468 was safe and well tolerated in toxicological studies by the subcutaneous and intravenous route in rodent and non rodent species. • AIC468 has shown a favorable pharmacokinetic profile with rapid distribution from the systemic circulation into tissues. • 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 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	

Table 1. Summary of preclinical safety studies with AIC468

Toxicology: body weight

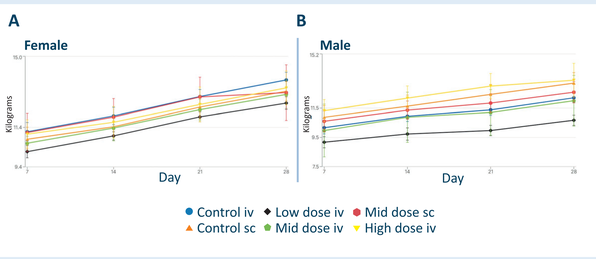


Figure 5. 4-week GLP toxicity in minipigs. 5 weekly doses iv and sc have been well tolerated. (A) No AIC468-related effects on body weight were noted for iv or sc treated females at all dose levels over the dosing period. **(B)** No AIC468-related effects on body weight were noted for iv or sc treated males at all dose levels over the dosing period.

Toxicology: Kidney and Liver

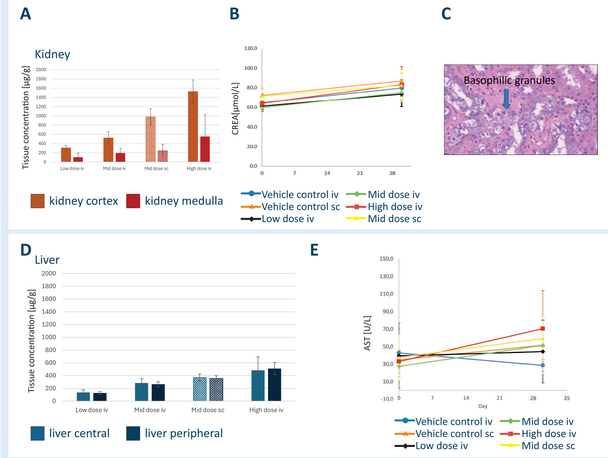


Figure 6. 4-week GLP toxicity study in minipigs demonstrated good tolerability.
(A) AIC468 accumulation in kidney. Increase was less than dose proportional, probably due to saturable uptake mechanism. Cortex concentrations 2-6-fold higher than medulla concentrations. Cortex concentration after sc administration 2-fold higher when compared to iv (most likely reason: saturable uptake mechanism, slower compound delivery after sc injection).
(B) Clinical chemistry (creatinine). No relevant changes in creatinine concentration compared to concurrent control groups have been observed.
(C) AIC468 accumulation in minipig kidney tubular epithelial cells. 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 AIC468 and are considered non-adverse*.
(D) AIC468 accumulation in liver. Liver concentrations were generally lower than kidney cortex concentrations. Overall, liver and kidney concentrations showed higher exposure after sc dosing compared to same dose given iv. No difference between central and peripheral liver lobe.
(E) Clinical chemistry (aspartate aminotransferase). A reversible increase in aspartate aminotransferase activity was noted at high dose iv when compared to the control group. AST declined to baseline after dosing was stopped.

*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.

Predicted therapeutic window

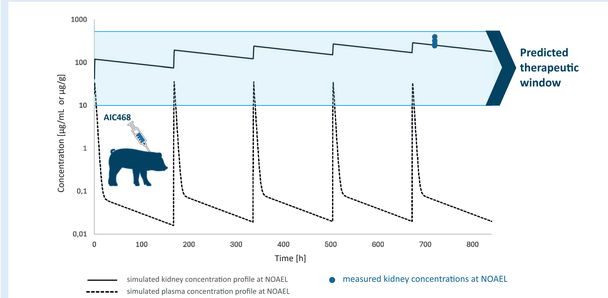


Figure 7. Broad predicted therapeutic window of AIC468 based on preclinical data.
PK data from 4-week minipig experiment revealed that efficacious concentrations in kidneys can be reached with low doses of AIC468 and support a weekly or less frequent dosing regimen. The efficacious concentration range in human kidney tissue (10-70 µg AIC468/g kidney cortex) was estimated from *in vitro* EC50 cell culture data (intracellular concentration). The upper limit of the therapeutic window was based on tissue concentrations which showed first signs of reversible toxicity.

Conclusions and outlook

- AIC468 is a novel, first in class, direct acting antiviral antisense oligonucleotide with potent anti-BKV activity and a favorable PK and toxicity profile
- Available data support the further clinical development of AIC468 for the treatment of BKV infections in kidney transplant recipients
- A first in human phase 1 single- and multiple-ascending dose study in healthy volunteers is approved and scheduled in Q3 2024