Preclinical Characterization of MAU868, a Novel Neutralizing Antibody Targeting BK Virus

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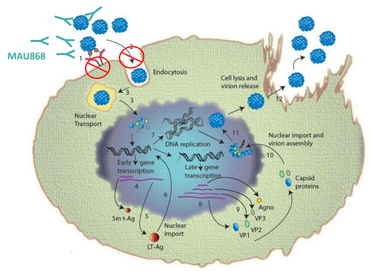
Introduction

- BK virus (BKV) is one of 13 known human polyomaviruses. Infection is essentially ubiquitous (estimated seroprevalence 80-90% of adults).
- Primary infection occurs during childhood and is usually asymptomatic or associated with mild, non-specific, upper respiratory symptoms.
- Persistent infection is established in the epithelial cells of renal tubules, ureters, and bladder but effectively controlled by the immune system.
- Compromised immune function can lead to uncontrolled BKV replication and development of disease, the best characterized of which is BKV nephropathy (BKVN).
- BKVN is a leading cause of early allograft loss in kidney transplant recipients.
- The need for specific and effective anti-BKV therapies remains unmet.

MAU868

- A novel, human monoclonal antibody currently in clinical development that binds to the BKV major capsid protein (VP1).
- pM binding affinity and sub-nM neutralizing potency against the four major genotypes of BKV (pan-serotype activity).
- High barrier-to-resistance *in vitro.*
- Neutralizing activity against the closely related JC virus, the cause of progressive multifocal leukoencephalopathy.

Figure 1. MAU868 mechanism of action



MAU868 disrupts binding of BKV to the host cell receptor and prevents entry and infection of new cells.

Methods

- MAU868 (human IgG1/λ targeting BKV VP1) was identified and isolated from human memory B cells from a healthy, BKV-seropositive donor.
- Binding affinity to BKV VP1 pentamers was determined using a solution equilibrium titration (SET) assay.
- Ability of MAU868 to neutralize BKV infection of primary renal proximal tubule epithelial (RPTE) cells was evaluated by quantitating BKV-infected (TAg-positive) cells using an immunofluorescence-based high content imaging (HCI) assay.
- *In vitro* barrier-to-resistance studies to investigate the emergence of resistance-associated variants with reduced susceptibility to MAU868:
  - Study 1: BKV genotype I and IV was serially passaged in RPTE cells in the presence of MAU868 (ranging from 0.5- to 10-fold over EC_{90}) for a total of 84 days.
  - Study 2: BKV (genotype I and IV)-infected HEK-293 cells were subdivided weekly in the presence of MAU868 (ranging from 0.5- to 5-fold over EC_{90}) for a total of 182 days.
- X-ray crystallography was employed to generate an atomic resolution structure of the MAU868 single-chain variable fragment (scFv) bound to BKV genotype I VP1 pentamers, solved at 2.66 Å resolution.

Results

MAU868 potently neutralizes all four major BKV genotypes *in vitro*

- MAU868 has picomolar binding affinity for BKV VP1 and sub-nanomolar neutralization potency against the four major BKV genotypes (Table 1).
- No evidence of cytotoxicity up to the highest concentration tested (500 µg/ml).
- MAU868 also neutralized infection by JC virus, a related human polyomavirus.
  - Mean EC_{50} of 0.215 µg/ml (COS-7 cells) and 0.056 µg/ml (SVGp12 cells) using an immunofluorescence-based HCI assay to quantitate JCV-infected (VP1-positive) cells.

Table 1. *In Vitro* Binding and Neutralizing Activity of MAU868 Against BKV

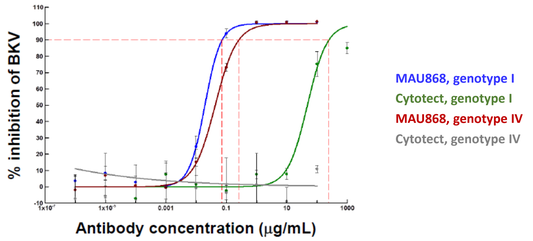
BKV Genotype	K_D	EC_{50}		EC_{90}	
	pM	µg/ml	nM	µg/ml	nM
I	5.8 ± 1.8	0.009 ± 0.010	0.062 ± 0.068	0.102 ± 0.028	0.708 ± 0.196
II	2.8 ± 0.6	0.040 ± 0.025	0.278 ± 0.175	4.160 ± 6.076	28.865 ± 42.159
III	8.4 ± 3.7	0.093 ± 0.057	0.645 ± 0.397	2.662 ± 2.805	18.473 ± 19.462
IV	4.1 ± 1.3	0.021 ± 0.020	0.143 ± 0.135	0.465 ± 0.318	3.229 ± 2.204

Equilibrium dissociation constant (K_D) was determined using a SET assay.
Neutralizing activity is expressed as the mean EC_{50} or EC_{90} ± standard deviation from three independent experiments.

MAU868 demonstrates higher potency compared with pooled intravenous immunoglobulin (IVIG) preparations

- IVIG preparations (eg, Cytotect) have been shown to contain neutralizing antibodies to BKV, but clinical efficacy in patients with BK viremia or BKVN has not been consistently demonstrated.
- Cytotect showed no neutralizing activity against BKV genotypes II, III, or IV up to the highest concentration tested (1000 µg/ml).
- MAU868 was 9,455-fold more potent against BKV genotype I than Cytotect.

Figure 2. *In Vitro* Neutralizing Activity of MAU868 Compared with Cytotect



MAU868 has a high *in vitro* barrier-to-resistance

Study 1:

- Virus replication (BKV genotype I or IV) was not detected in the presence of any MAU868 concentration over the course of 84 days.
- In parallel, viral breakthrough was detected in the presence of a control anti-BKV VP1 antibody after 14 days (genotype IV) and after 42 days (genotype I). Sequence analysis identified VP1 mutations that conferred reduced susceptibility to the control antibody but not to MAU868.

Study 2:

- Virus replication was not detected in BKV genotype IV infected HEK-293 cultures over the course of 182 days.
- BKV genotype I infected HEK-293 cultures: virus replication was detected in the presence of lower MAU868 concentrations, but no VP1 mutations were identified and the resulting virus remained susceptible to MAU868.
  - No virus replication was detected in the presence of higher MAU868 concentrations out to 182 days.

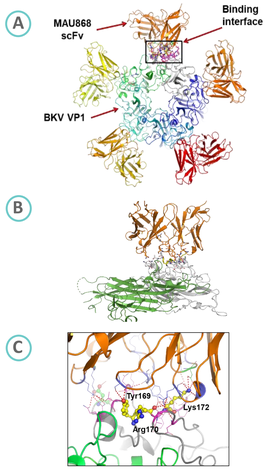
MAU868 neutralizes infection by clinically relevant BKV variants

- MAU868 demonstrated potent neutralization activity against BKV variants constructed to contain intact VP1 sequences from frequently reported clinical isolates (Table 2) or highly prevalent polymorphisms in the BC loop (major receptor binding loop; residues 57-89) of VP1 with no shift in potency.

Table 2. *In Vitro* Neutralizing Activity of MAU868 Against BKV Variants

BKV Genotype	GenBank Accession No.	GenBank Entries (No.)	Amino Acid Change (relative to the reference isolate)	MAU868 EC_{50} (µg/ml)
I	BAF42997	4	D175E	0.014 ± 0.008
I	BAI43630	9	E20D, D175E, V210I, R340K	0.006 ± 0.010
I	BAI43624	72	E20D, D175E, V210I, F225L, R340K	0.007 ± 0.010
I	AEO89604	20	V42L, E82D, D175E, V210I	0.006 ± 0.009
I	AEO89592	21	V42L, D175E, V210I, L362V	0.033 ± 0.040
I	CBX88362	34	V42L, D175E, V210I, R340K	0.008 ± 0.006
I	CAA24307		**Reference isolate MM**	**0.024 ± 0.017**
IV	BAG75403	10	D77E	0.003 ± 0.002
IV	BAG75223	8	I121M	0.016 ± 0.005
IV	BAG75259	9	E138D, Q340K	0.022 ± 0.016
IV	BAG75415	16	I145V	0.0001 ± 0.00005
IV	BAF75132		**Reference isolate ITA-4**	**0.066 ± 0.090**

Figure 3. Crystal structure of MAU868 scFv bound to BKV VP1 pentamers



Each VP1 monomer (colored green, teal, light blue, dark blue, and gray) within the VP1 pentamer has a single isolated binding site for MAU868 scFv (colored yellow, red, and orange).

MAU868 scFv bound to the epitope, composed of an interface between two adjacent BKV VP1 monomers.

Magnification of the contacts between MAU868 scFv and the VP1 pentamer, showing interactions with the three key contact residues on VP1: Y169, R170, and K172.

Conclusions

- The highly conserved key contact residues within the conformational epitope of VP1 that is recognized by MAU868 may explain the broad-spectrum neutralizing activity and high *in vitro* barrier-to-resistance.
- MAU868 is a promising, novel, human anti-BKV monoclonal antibody with the potential to be the first specific therapy for the treatment and/or prevention of BKV disease.

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