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14. ABSTRACT The goal of this proposal is to develop and establish preclinical proof-of-concept for the first universal therapeutic antibodies that protect against disseminated candidiasis caused by all the medically important Candida species. A panel of functional monoclonal antibodies (mAbs) protect immunocompetent and immunocompromised mice against disseminated candidiasis have been identified. Uniquely, these protective mAbs target three universal peptide epitopes (UP1, UP2 and UP3) expressed 91%-100% by all the medically important Candida species, including C. auris. Furthermore, immunocompromised murine models of disseminated infection by C. tropicalis, C. glabrata, C. parapsilosis and C. krucei have been successfully established, which closely mimic the immunocompromised patient situation in clinical settings. These mouse models will be used in proposed study and prove valuable for evaluating therapies to control Candida infections. The hypothesis to be tested is that novel mAb-based therapy would 1) target multiple antigenic epitopes to achieve the greatest efficacy as compared to single mAb treatment, and 2) be composed of epitopes that are homologous among medically relevant species of Candida such that universal protection against Candida infection could be achieved, and 3) have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis.

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1. INTRODUCTION:

Disseminated candidiasis is a life-threatening disease and a leading cause of bloodstream infections afflicting immunocompromised and hospitalized patients in the United States. Given the high mortality rate and significant burden on the healthcare system associated with disseminated candidiasis, novel approaches are needed to supplement or replace current antifungal therapy. The goal of this proposal is to develop and establish preclinical proof-of-concept for the first universal therapeutic antibodies that protect against disseminated candidiasis caused by all the medically important *Candida* species. Three specific Aims were designed to achieve the goal. First, a combination therapy with mAb cocktails for disseminated candidiasis by *C. albicans,* the most common disease-causing species (65%), were established. Different mAb combinations were further tested. Secondly, the protective efficacy of therapeutic mAb cocktails in immunocompromised mouse models of disseminated candidiasis by *non-albicans Candida* species were determined. Finally, the mAbs that have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis will be identified.

2. KEYWORDS:

- Disseminated candidiasis
- *Candida albicans*
- *C. tropicalis*
- *C. glabrata*
- *Non-albicans Candida (NAC)*
- *Candida auris*
- Immunotherapeutic
- Monoclonal antibody-based therapy
- Immunocompromised
- Cyclophosphamide (CY)
- Fluconazole (FLC)
- Amphotericin B (AMB)
- Colony forming unit (CFU)

3. ACCOMPLISHMENTS

What were the major goals of the project?

The goal of this proposal is to develop and establish a preclinical proof-of-concept for the first universal therapeutic antibodies that protect against disseminated candidiasis caused by all of the medically important *Candida* species. In three Aims, we demonstrated **1,** therapies using a combination of protective mAbs can provide synergy in the protection against disseminated candidiasis caused by *C. albicans* in mice. **2,** we further validated the therapeutic mAb cocktails identified in Aim 1 can provide enhanced protection against disseminated candidiasis cause by *NAC* in immunocompromised mice. **3**, we will develop novel antifungal therapies with mAbs in combination with antifungal agents in Aim 3.

Below is the completion of major goals as stated in the approved SOW

Specific Aim 1	**Timeline**	**Status**
Major Task 1: Develop combination therapy with mAb cocktails for disseminated candidiasis by *C. albicans*		Achieved 100%
Subtask 1: Produce and purify mAbs and test functional titers of mAbs	1	Achieved 100%
Subtask 2: Evaluate the therapeutic efficacy of the mAb cocktails in mouse model of disseminated candidiasis by *C. albicans*	2-4	Achieved 100%
Subtask 3: Further evaluate whether different ratios of the two mAbs in cocktails can change the therapeutic efficacy in mouse model of invasive candidiasis	5-6	Achieved 100%
Milestone(s) Achieved	6	
Milestone 1: Develop an effective therapeutic antibody treatment against disseminated candidiasis caused by *C. albicans,* and even protect against antifungal resistance in multiple *C. albicans strains*	6	Achieved 100%
Specific Aim 2		
Major Task 2: Determine the protective efficacy of therapeutic mAb cocktails in immunocompromised mouse models of disseminated candidiasis by *non-albicans (NAC) Candida* species		Achieved 70%
Subtask 1: Establish and maintain immunocompromised mouse models of invasive *NAC* infection	7-8	Achieved 80%
Subtask 2:Evaluating therapies of mAb cocktails against disseminated *NAC* infection	7-9	Achieved 80%
Subtask 3: Troubleshoot and adjust ratios of mAbs to achieve best efficacy.	10-12	Achieved 50%
Milestone(s) Achieved:	12	70%

Milestone 2: Develop a broad-spectrum mAb therapies protect immunocompromised host against the medically important *Candida* species	12	Achieved 70%

Specific Aim 3		To be performed
Major Task 3: Determine whether the mAbs have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis.		To be performed
Subtask 1: Test sub-therapeutic / full dose of mAb, FLC and AMB	13-15	
Subtask 2: examine the combination therapy of each mAb/ mAb cocktails with fluconazole (FLC) and AMB	16-18	
Milestone(s) Achieved:	18	
Milestone 3: Develop mAb therapies used as adjuncts to current antimicrobial therapy, to improve therapeutic efficacy in immunocompromised host		

What was accomplished under these goals

Aim 1: To develop combination therapy with mAb cocktails for disseminated candidiasis by *C. albicans*

1) Major activities

Based on our identified universal peptide (UP)-related mAbs, combination therapy composed of two mAbs specific for two different UP epitopes, which were derived from two different cell wall proteins, were tested for enhanced protection against the disease as compared to a single mAb treatment. The best therapeutic mAb cocktails were determined. The goal of this Aim is to show that immunotherapies using a combination of mAbs can provide enhance or synergy in the protection against disseminated candidiasis caused by *C. albicans* in immunocompetent mice (B6, BALB/c and A/J).

2) specific objectives

The therapeutic efficacy of the mAb cocktails were first tested in a BALB/c mouse model of disseminated candidiasis, as described before. A minimal dose of each mAb that provides a sufficient protection, defined in the preliminary study, as used: 2C9 (0.2μg/μl, titer 80,000), 5A9

(0.25µg/µl, titer 80,000), 6E3 (0.3µg/µl, titer 100,000) and 10E7 (0.35µg/µl, titer 100,000). Mice were intravenously (i.v.) challenged with a lethal dose of live *C. albicans* (SC5314, ATCC MYA-2876) yeast cells 4 hours prior to the administration of a single dose of each mAb or mAb cocktail (Table 1). Controls included mice that have received each individual mAb alone, or mice that have received an irrelevant mouse mAb isotype control, isotype control mAb cocktail, or DBPS buffer (Table 1).

Table 1. Experimental design of tested mAb cocktails and control groups in Aim 1

Therapeutic mAb cocktails	Control #1	control #2	control #3	control #3
2C9 + 6E3	2C9	6E3	Irrelevant mouse IgG2a	Irrelevant mouse IgG1
5A9 + 6E3	5A9	6E3	Irrelevant mouse IgG3	Irrelevant mouse IgG1
2C9 + 10E7	2C9	10E7	Irrelevant mouse IgG2a	Irrelevant mouse IgG1
5A9 + 10E7	5A9	10E7	Irrelevant mouse IgG3	Irrelevant mouse IgG1
6E3 + 10E7	6E3	10E7	Irrelevant mouse IgG1	Irrelevant mouse IgG1

Animal model: 5-7 week old BALB/c female and male mice

13 groups / 5 mice per group; repeated 3X

1. mice- mAb recipients **(2C9 + 6E3)**
2. mice- mAb recipients **(5A9 + 6E3)**
3. mice- mAb recipients **(2C9 + 10E7)**
4. mice -mAb recipients **(5A9 + 10E7)**
5. mice- mAb recipients **(6E3 + 10E7)**
6. mice- mAb recipients **(2C9)**
7. mice- mAb recipients **(5A9)**
8. mice- mAb recipients **(6E3)**
9. mice- mAb recipients **(10E7)**
10. mice- mAb recipients **(Irrelevant mouse IgG2a)**
11. mice- mAb recipients **(Irrelevant mouse IgG3)**
12. mice- mAb recipients **(Irrelevant mouse IgG1)**
13. mice- DPBS control

3) Significant results and key outcomes

Several combinations of mAbs (2C9+6E3, 5A9+6E3 and 5A9+10E7) were demonstrated to be more effective in protecting against disseminated candidiasis as compared to each mAb alone in BALB/c mouse model of disseminated candidiasis (Table 2). The protection was evidenced by a significantly increased survival rate by day 60 post-infection and reduced or non-detectable fungal burden (Colony forming units, CFUs) in kidneys. Three out of five mAb cocktails showed an enhanced / synergistic effect, which represented a critical improvement in mAb therapy. With this

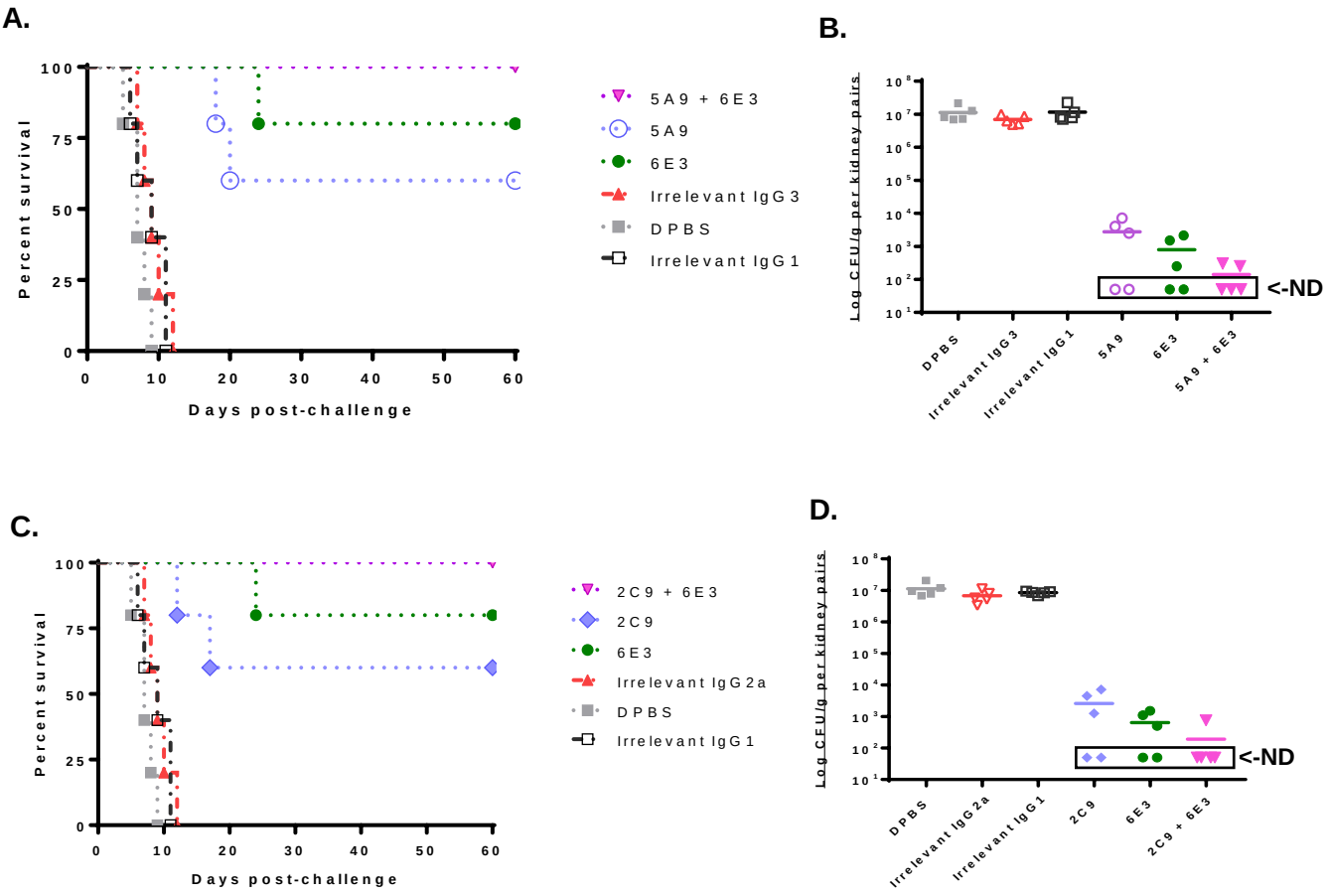
supporting data, therapeutic combinations that include 2-3 mAbs in the form of different isotypes and epitope specificities may be further designed, since a cocktail of functional mAbs could provide more protection and target more microbial strains than a single mAb.

Table 2. Combined two universal-peptide–specific mAbs (UP related mAb cocktails) conferred synergy / enhanced protection against systemic candidiasis by passive transfer to naïve mice as compared to single mAb treatment.

Protective mAb cocktails	Control #1	control #2	Protection of mAb cocktails	Synergy of combination
2C9 + 6E3	2C9	6E3	High (90-100%)	**Yes++**
5A9 + 6E3	5A9	6E3	High (80-100%)	**Yes++**
2C9 + 10E7	2C9	10E7	Moderate (60-80%)	No
5A9 + 10E7	5A9	10E7	High (100%)	**Yes++**
6E3 + 10E7	6E3	10E7	High (70-80%)	No

Aim 1: Survival and CFU data

Treatment with two mAbs in combinations leading to synergy in protection



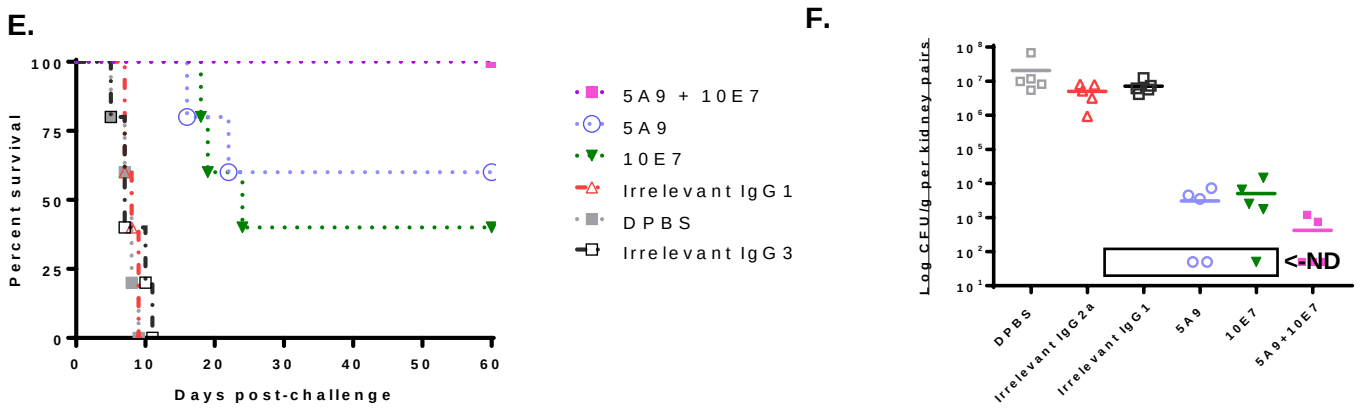
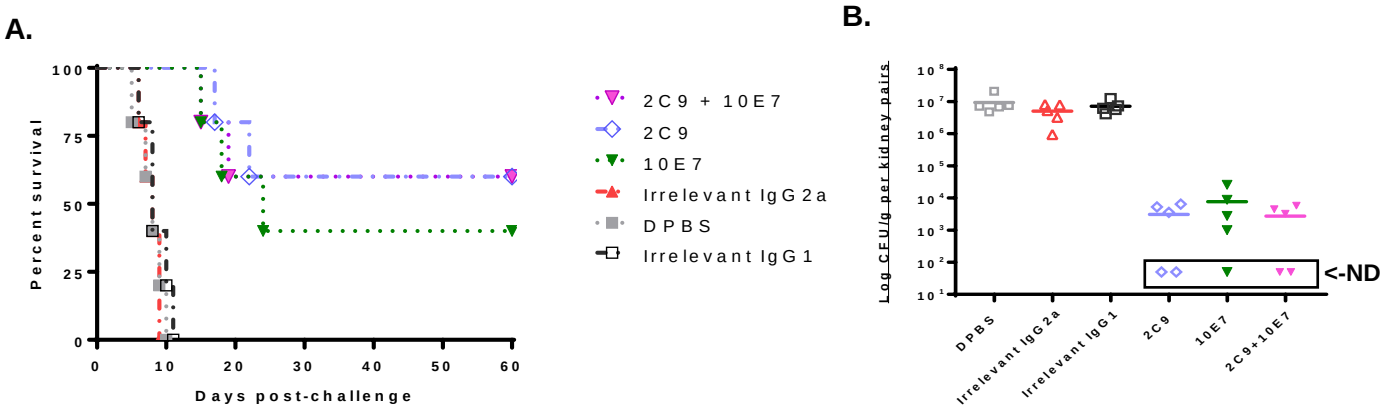


Figure 1: Two protective mAbs in combination conferred best protection against systemic candidiasis in passive transfer experiments. A. Identified protective mAbs listed in Table 2 were obtained by use of standard hybridoma technique and purified by use of protein A/G. BALB/c mice were given an i.p. dose of mAb 5A9 (IgG3, specific for UP1 peptide vaccine), or mAb 6E3 (IgG1, specific for UP2 peptide vaccine) or the mAbs in combination (5A9 + 6E3) 4 hours before hematogensous challenge with a lethal dose ($5x10^5$) of *C. albicans* SC5314 live cells. Mice received either 5A9 or 6E3 had 80% or 60% survival rate respectively, with prolonged survival as compared to control animals that received either DPBS or irrelevant mAb isotype control IgG3 or IgG1. The two mAbs combination (5A9+6E3) treatment was able to provide the best protection, 100% survival up to 60 days. **B.** Consistently, the group received treatment of two protective mAbs in combination (5A9+6E3) had the least CFUs among all the groups ($P<0.01$), 3 out of 5 survivors have non-detectable (ND) CFU in kidney, indicating some survivors were able to clear up the fungal burden from the targeted organ-kidney. The non-detectable CFU in groups received mAb(s) treatment provided strong additional evidence for the protection being due to the mAb/mAb cocktail treatment. **C.** The same synergy in protective efficacy was also observed in group received treatment of mAb 2C9 (mAb IgG2a, specific for UP1 peptide vaccine) and 6E3 (IgG1, specific for UP2 peptide vaccine) in combination-100% survival up to 60 days. **D.** Consistently with survival data, the group received treatment of the mAbs in combination (2C9+6E3) had the least CFUs among all the groups ($P<0.01$), 4 out of 5 survivors have non-detectable (ND) CFU in kidney, and only one mouse survivor had detectable low CFUs in kidney. **E.** Mice received single mAb10E7 (IgG1, specific for UP3 peptide vaccine) treatment had 40% survival, and mice received mAb 5A9 alone had 60% survival up to 60 days post-infection. The two mAbs combination (5A9+10E7) treatment was able to provide the best protection, 100% survival up to 60 days. **F.** Consistently, the group received treatment of two protective mAbs (5A9+10E7) had the least CFUs among all the groups ($P<0.01$), 3 out of 5 survivors have non-detectable (ND) CFU in kidney. Statistical evaluations were performed using Prism 7.0 (GraphPad). Survival times were statistically evaluated by Kaplan–Meier (GraphPad Prism, version 7). Fungal burden in organs as determined by CFU counts per gram was analyzed with Mann-Whitney's non-parametric test when two groups were compared.

Treatment with two mAb in combination has the comparable protective efficacy as the each single mAb treatment, no synergy was observed.



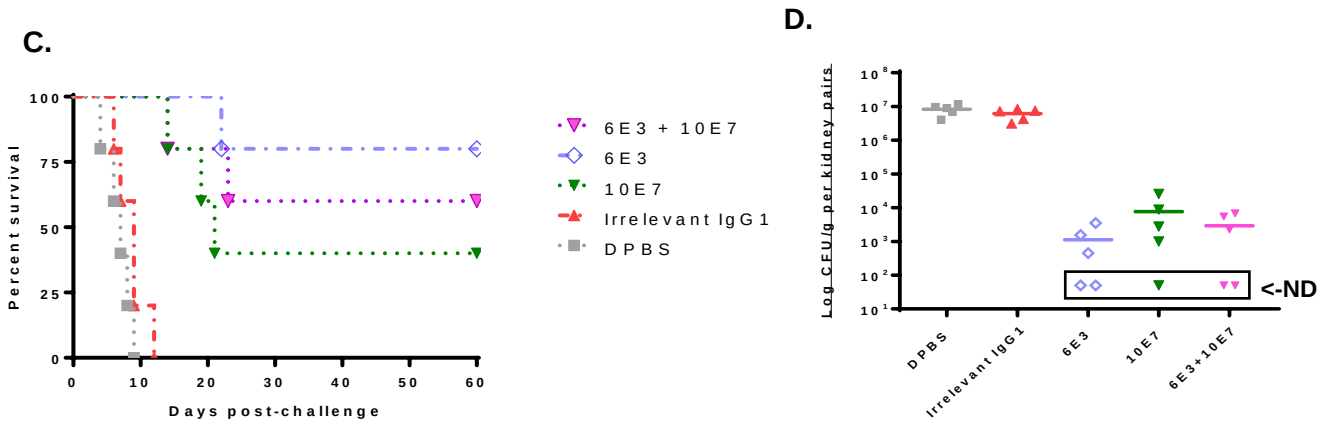


Figure 2: Two protective mAbs in combination conferred the comparable protection as compared to single mAb treatment against systemic candidiasis in passive transfer experiments. A. BALB/c mice were given an i.p. dose of mAb 2C9 (mAb IgG2a, specific for UP1 peptide vaccine) or mAb 10E7 (IgG1, specific for UP3 peptide vaccine) or the mAbs in combination (2C9 + 10E7) 4 hours before hematogensous challenge with a lethal dose ($5x10^5$) of *C. albicans* SC5314 cells. Mice received either 2C9 or 10E7 had 60% or 40% survival rate with prolonged survival as compared to control animals that received either DPBS or irrelevant mAb isotype control IgG2a or IgG1. The two mAbs combination (2C9+10E7) treatment provided the comparable protection as compared to single 2C9 treatment, 60% survival up to 60 days. No synergy was observed with two mAb cocktail as compared to single mAb in efficacy. **B.** the group received treatment of two protective mAbs in combination (2C9+10E7) had the similar CFUs with single mAb treated groups. In either 2C9 or 2C9+10E7 group, 2 survivors had non-detectable (ND) CFU in kidney, indicating some survivors could clear up the fungal burden from the targeted organ-kidney. **C.** No enhanced protection was observed in group received mAb cocktail (6E3+10E7) treatment as compared to the group treated with mAb 6E3 alone. **D.** Consistently with survival data, there is no significant difference between the group received treatment of two protective mAbs in combination (6E3+10E7) and groups treated with single mAb.

Aim 2: To determine the protective efficacy of therapeutic mAb cocktails in immunocompromised mouse models of disseminated infection by *non-albicans Candida* (*NAC*) species.

1) Major activities

Several immunocompromised / gene deficiency mouse models of invasive *NAC* infection, including *C. tropicalis*, *C. glabrata* and *C. auris* has been successfully established by us (Xin H, Med Mycol. 2018 Dec 6). These mouse models have been a valuable tool for evaluating therapies of mAb cocktails against disseminated *NAC* infection.

Additionally, one critical challenge in human clinical situations is that antifungal treatments are not as effective in immunocompromised hosts as antimicrobial therapy used in hosts with intact immunity. The goal of Aim 2 has been especially focused on determining whether the therapeutic mAb cocktails identified in Aim 1 can provide the same enhanced protection against disseminated candidiasis in immunocompromised hosts, which mimic high-risk population in clinical situation who are highly susceptible to invasive candidiasis.

2) Specific objectives

We have demonstrated and reported that some mAbs protect neutropenic mice against disseminated candidiasis. Experiments in Aim 2 have been focused on testing the enhanced therapeutic efficacy of the mAb cocktails in immunocompromised mouse model of disseminated candidiasis caused by three clinically significant *NAC* species, *C. tropicalis, C glabrata* and *C. auris*

(*C. auris* was reported by CDC since 2009 as multi-drug resistant superbug). Animals were rendered into an immunocompromised state and maintained for 50 days by cyclophosphamide (CY) i.p. and/or cortisone s.c. as given on day -3 (Xin H, *Med Mycol*) (Fig. 3). On day 0, mice of different experimental groups (10 mice per group) were intravenously infected with a lethal dose of each of the *Candida* strains (Table 3). For therapeutic studies, we follow the same experimental approach and group design in Aim 1. In brief, three best protective mAb cocktails identified in Aim 1 were given 4h post-infection. Mice treated with each single mAb was used as positive controls to compare the therapeutic efficacy with mice treated with mAb cocktails. There is growing evidence supporting mAbs, of the appropriate isotype and specificity, as being protective against various forms of candidiasis in immunocompromised mice; the same enhanced efficacy from the use of two mAb cocktails in immunocompromised mice as that in immunocompetent mice were demonstrated in studies of Aim 2.

3) Significant results and key outcomes

We have identified two combinations of mAbs (2C9+6E3 and 5A9+10E7) to be more effective in protecting in our established immunocompromised mouse models of disseminated candidiasis caused by *C. tropicalis* or *C. glabrata*, as compared to single mAb treatment. The protection was evidenced by a significantly increased survival rate by day 50 post-infection as well as significantly reduced or non-detectable fungal burden (CFUs) in kidneys (Figure 1 +2). The two-mAb cocktails with synergistic protective efficacy represented a critical improvement in mAb-based therapy, not only aim to target for *C. albicans*, but for other medically significantly *Candida* species. With this supporting data, therapeutic combinations that include 2-3 mAbs in the form of different isotypes, and epitope specificities will be further designed and tested for the potential to provide broader protection, and target more microbial virulence factors.

a. **We have successfully established murine models of disseminated infection by *C. tropicalis*, *C. glabrata* and *C. auris.***

C. albicans is the most common disease-causing species (65%); however, the prevalence of disease caused by *non-albicans Candida* (*NAC*) species is on the rise, along with an increase in antifungal drug resistance. *C. glabrata*, now accounts for approximately 15 to 20% of all *Candida* infections in the United States, is the most common *NAC* species isolated. A particular problem with *C. glabrata* is its resistance to the most common drug classes, azoles and echinocandins. Another *NAC* species, *C. tropicalis*, in particular, is associated with invasive infection, accounting for up to 45% of *NAC* infections. Knowledge of immunity to *NAC* species is still at an early stage due to the lack of tractable animal models with which to study these important pathogens. This is partly because many *NAC* species are not usually pathogenic in mouse models of candidiasis. To study these clinically important pathogens, we successfully established immunocompromised murine models of disseminated infection by *C. glabrata* and *C. tropicalis* (Xin H, *Med Mycol.* 2018 Dec 6), as well as naïve mouse model for *C. auris* invasive infection (Table 3). For disseminated *C. tropicalis* infection, a combination of a large inoculum and immunosuppression was needed to establish severe acute infection of in both BALB/c mice and C57BL/6. However, this strategy was not a prerequisite for *C. glabrata* disseminated candidiasis in the intravenous mouse model of both strains. To establish a simple and more reliable intravenous mouse model for acute infection of *C. glabrata,* the C5-deficient A/J strain was used to evaluate *C. glabrata* virulence. Eventually, we established cyclophosphamide (CY)-treated immunosuppressed A/J intravenous model to be an

appropriate model for human disseminated *C. glabrata* infection, mimicking patients who develop deficiencies, including neutropenia. Most recently, we also established an inbred naïve A/J mouse model of systemic *C. auris* infection without immunosuppression (manuscript submitted to mSphere), to study this little know host-pathogen interaction.

In summary, our preliminary studies determined the optimal dose of each *Candida* strain for producing an acute infection, with 80-100% of animals dying within 7-14 days in different mouse strains (Table 3). The successfully established immunocompromised/gene deficient mouse models closely mimic the immunocompromised patient situations in clinical settings, and become a valuable tool as well as the foundation for evaluating mAb-based therapies to control *Candida* infections.

Table 3. Intravenous inoculum size of *Candida* strains in BALB/c and A/J mouse models

Candida strains	Challenge dose in immunocompromised mice	Mouse strain
C. albicans (ATCC36082)*	$5x10^4$ CFU	BALB/c
Candia tropicalis (ATCC200956)	$1x10^7$ CFU	BALB/c; A/J
C. glabrata (ATCC 200918)	$1 x10^8$ CFU	A/J
C. auris (CDC 0386)	$2x10^8$ CFU	A/J (w/o CY treatment)

Data of established murine models of disseminated infection by *C. tropicalis*, *C. glabrata* and *C. auris.* (Figure 3-7 for Table 3)

We have established an immunosuppressed mouse model of disseminated candidiasis by the two clinically important *NAC* species, *C. glabrata and C. tropicalis* (Figure 3-6)

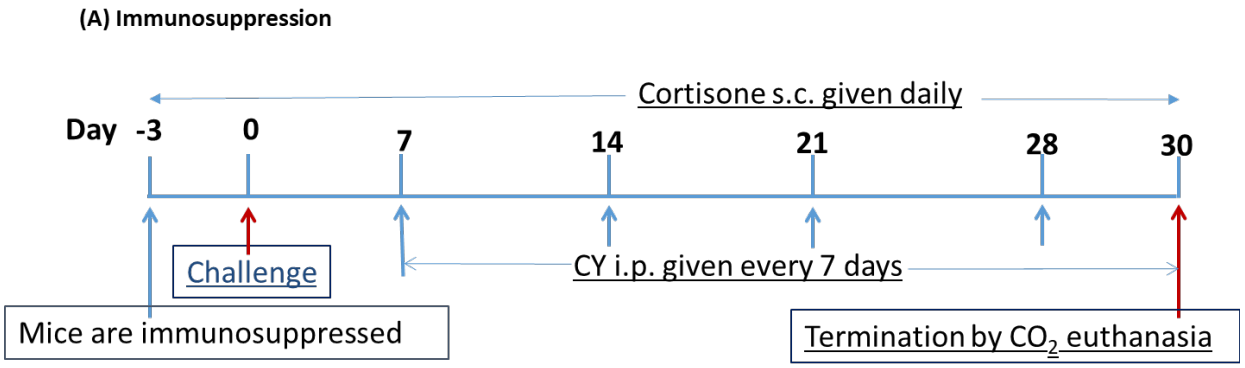
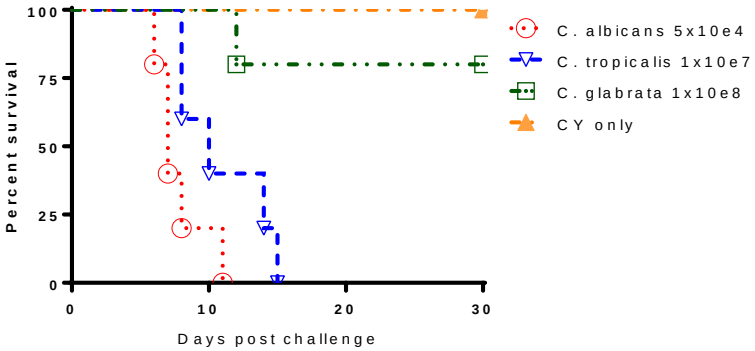


Figure 3. (A) Time course of the protocols for induction of immunosuppression. Before challenge, BALB/c and A/J mice were immunosuppressed by i.p. with CY or s.c. injection of cortisone acetate at day -3. The i.p. injection (150 mg/kg) of CY was repeated every 7 days after infection and s.c. injection of cortisone (125 mg/kg) was performed daily to maintain leukocytopenia.

- **Comparison of virulence of *C. glabrata* and *C. tropicalis* in immunosuppressed BALB/c and A/J mice and determined the optimal dose of each *Candida* strain for producing an acute infection, with 80-100% of animals dying within 7-14 days in different mouse strains (Figure 4).**

A.



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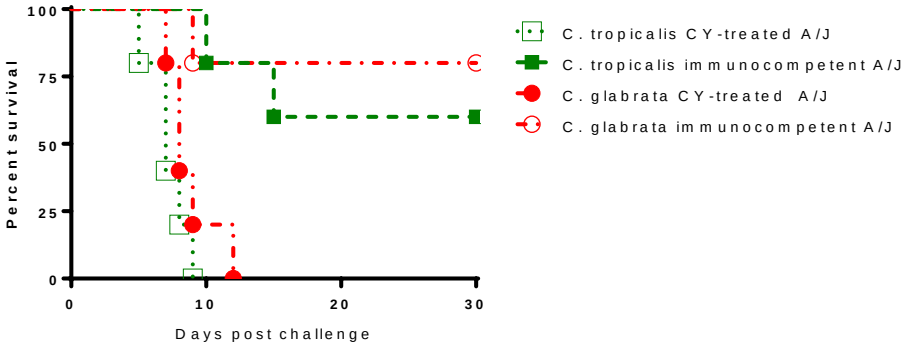

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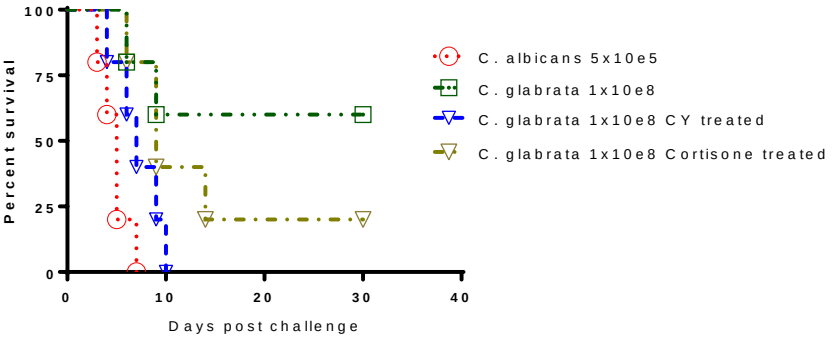


Figure 4. Establish reliable and optimal mouse strain for models of disseminated candidiasis by *C. tropicalis* and *C. glabrata*. A. BALB/c mice treated with cyclophosphamide is a feasible mouse model for disseminated candidiasis by of *C. tropicalis*. Naïve BALB/c mice received a 200 mg/kg dose of cyclophosphamide (CY) by i.p. on day -3. Prolonged immunocompromised status was maintained for 30 days by giving each animal a 150mg/kg dose of CY by i.p. every 7 days after infection. On day 0, mice of different experimental groups (5 mice per group) were intravenously infected with $1x10^8$ *C. glabrata* ATCC 200918 cells and $1x10^7$ *C. tropicalis* (ATCC200956) cells in 0.1 ml DPBS. As controls, immunocompromised BALB/c mice were also challenged with $5x10^4$ *C. albicans* SC5314 yeast cells as lethal dose. Survival data show

immunosuppressed BABL/c mice are more susceptible to the intravenous inoculum of 10^7 *C. tropicalis*, 100% mortality was achieved within 15 days post infection. However, even CY-treated BALB/c mice were still resistant against systemic infection by *C. glabrata*, only 20% mortality was observed within 30 days post-infection. **B. A/J mice are more susceptible to disseminated candidiasis by both *C. glabrata* and *C. tropicalis* as compared to BALB/c.** A/J mice were immunosuppressed with 200 mg/kg dose of cyclophosphamide (CY) by i.p. on day -3. Prolonged neutropenia were maintained by the same CY regimen described as before. On day 0, both immunocompetent and immunosuppressed mice were intravenously infected with viable $1x10^8$ *C. glabrata* ATCC 200918 cells or $1x10^7$ of *C. tropicalis* ATCC200956 in 0.1 ml DPBS. As controls, immunocompetent A/J mice were challenged with the same dose of *Candida* cells as for immunosuppressed A/J mice. **C. Immunosuppressed A/J intravenous mouse model of *C. glabrata* disseminated infection.** A/J mice received either a 200 mg/kg dose of cyclophosphamide (CY) by i.p. or subcutaneous injections of cortisone acetate at a dose of 125 mg/kg on day -3. Prolonged neutropenia were maintained for 30 days by giving each animal a 150mg/kg dose of CY by i.p. every 7 days or 125 mg/kg dose of cortisone by s.c. daily after infection. On day 0, mice were intravenously infected with viable $1x10^8$ *C. glabrata* ATCC 200918 cells in 0.1 ml DPBS. As controls, immunocompetent A/J mice were challenged with the same dose of *C. glabrata* cells as for immunosuppressed A/J mice. *C. albicans* disseminated A/J mouse model was used as a positive control for *Candida* acute systemic infection.

- **Influence of immunosuppression on fungal burden and pathological alterations during the course of infection-dissemination and growth of *C. glabrata* in multiple targeted organs of immunocompetent and immunosuppressed BALB/c mice (a) and A/J mice (Figure 5).**

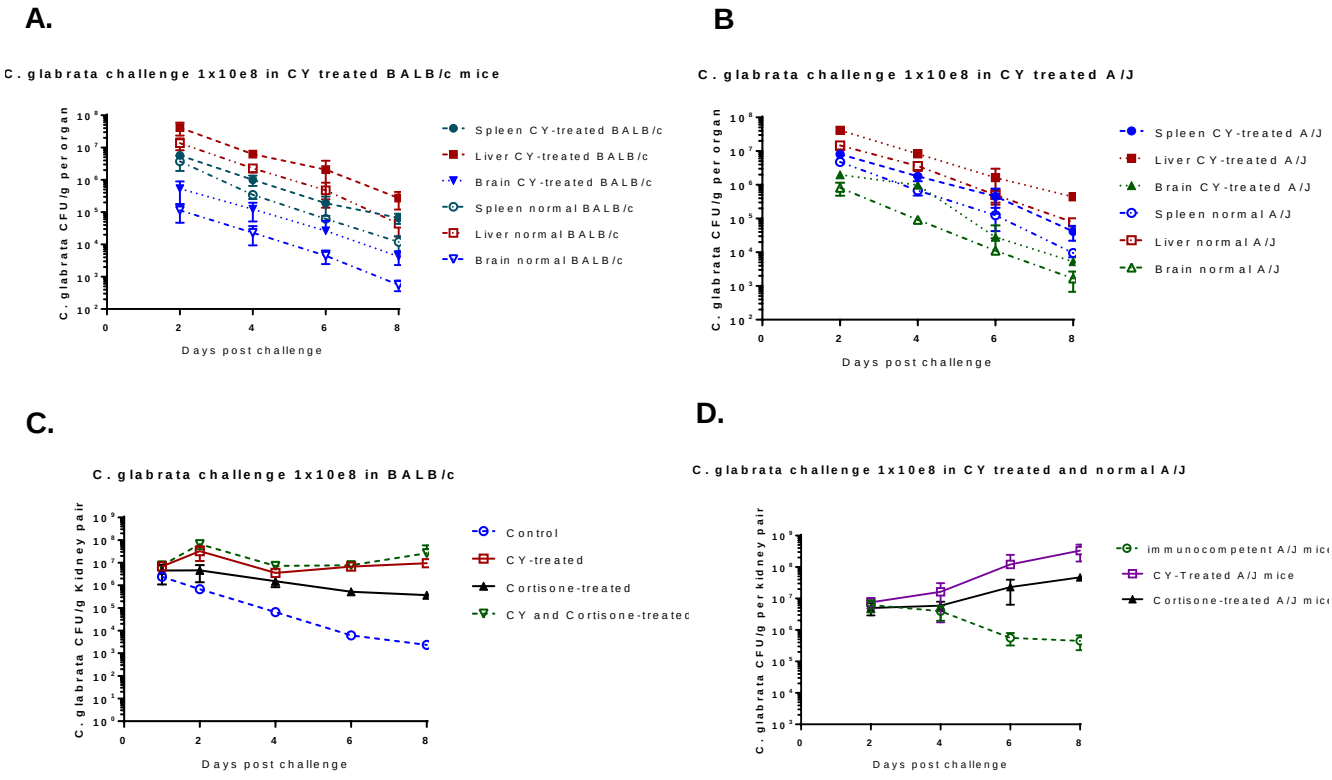
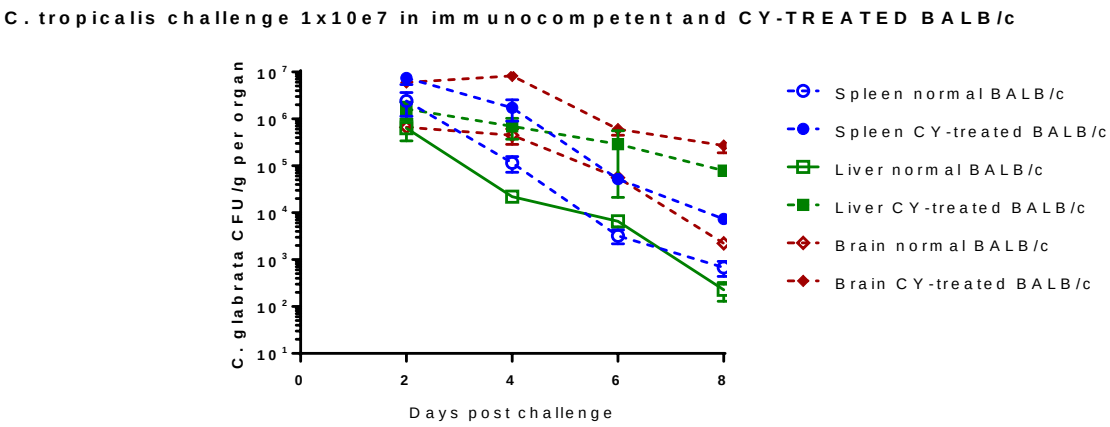


Figure 5. Influence of immunosuppression on fungal burden in multiple organs during the course of systemic *C. glabrata* infection. To compare the effect of immunosuppression on the outcome of *C. glabrata* systemic infection in both A/J and BALB/c mice, we treated 20 mice of each strain with CY regimen or left untreated (immunocompetent) on day -3. Both immunosuppressed and untreated mice were inoculated i.v. with *C. glabrata* ATCC 200918 (10^8) on day 0. At each specific time point post-infection (day 2, 4, 6 and 8), five mice were euthanized, and net growth of *C. glabrata* quantified and compared in spleen, liver and brain in BALB/c

mice **(A)** and A/J mice **(B)**. Results represent the mean SEM of CFU /tissue of five mice per time point. The CY-treated mice had significantly higher mean CFU in the organs examined than the normal group, and CY-treatment delayed fungus clearance in all tested organs of both mouse strains. Kidney is the most important targeted organ during invasive candida infection, mimic human clinical situations. At each specific time point post-infection (day 2, 4, 6 and 8), five mice were euthanized, and net growth of *C. glabrata* was quantified and compared in kidneys in BALB/c mice **(C)** and A/J mice **(D)**. Results represent the mean SEM of CFU /tissue of five mice per time point.

- **Dissemination and growth of *C. tropicalis* in tissues of immunocompetent and CY-treated immunosuppressed BALB/c mice (Figure 6).**

A.



B.

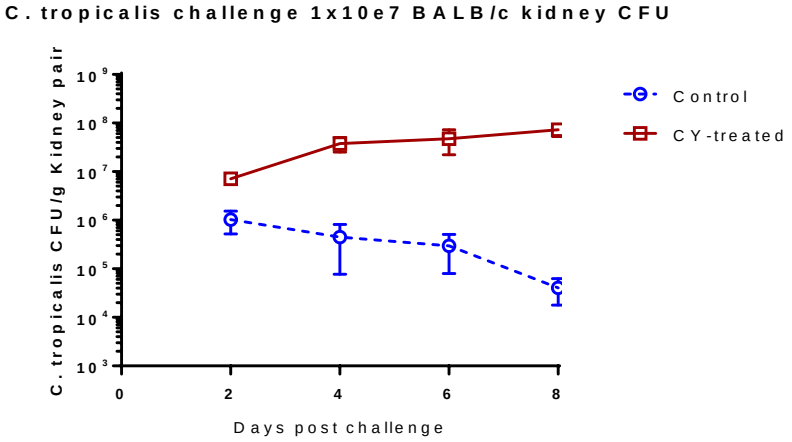


Figure 6. Dissemination and growth of *C. tropicalis* in tissues of immunocompetent and CY-treated immunosuppressed BALB/c mice. 20 naïve BALB/c mice were immunosuppressed by CY regime as describe before. Immunosuppressed BALB/c mice and age- and sex-matched normal control mice (*n* = 20 in each group) were inoculated i.v. with *C. tropicalis* (10^7 organisms/mouse) on day 0. At each specific time point post-infection (day 2, 4, 6 and 8), five mice were euthanized, and net growth of *C. tropicalis* was quantified in spleen, livers and brains **(A)** and kidneys **(B)** by culture of tissue homogenates. Results represent the mean SEM of CFU /tissue of five mice per time point. The results of CFU determinations of infected kidneys were consistent with those of mortality and number of CFU/g kidney tissue was significantly higher in CY treated mice *as* compared with those immunocompetent mice with same inoculum.

We have established an inbred A/J mouse model of systemic *C. auris* infection without immunosuppression (Figure 7)

After two independent experiments, we developed an immunocompetent inbred A/J mouse infection model by screening two different clinical isolates of *C. auris* (0381 and 0386) for their lethality in mice (data not shown). We focused on testing *C. auris* 0386 in all the challenge experiments due to extremely slow growth rate of C. auris 0381 strain. We compared the effect of immunosuppression on the outcome of *C. auris* systemic infection in A/J mice, and demonstrated all A/J mice, dispensable on the immunosuppression status, had high fungal burden in heart and kidney.

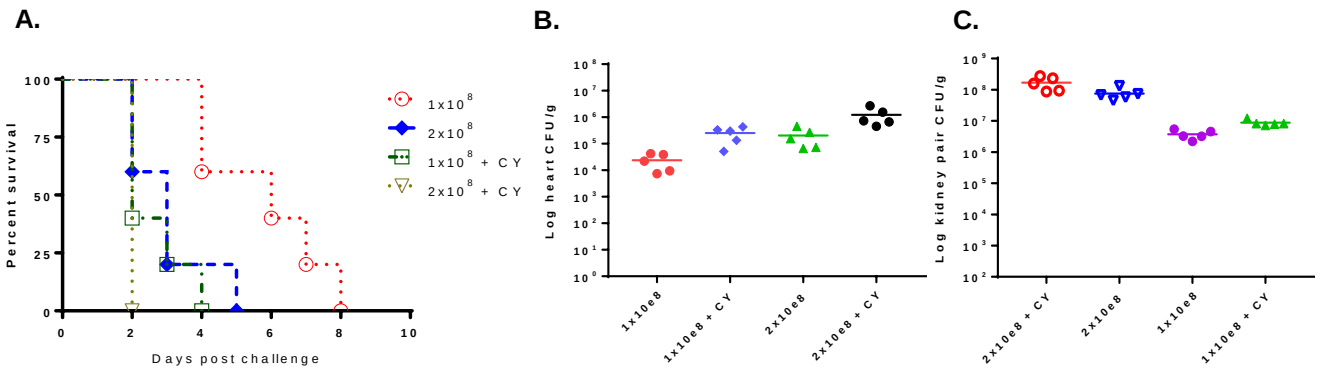


Figure 7. Evaluation of *C. auris* virulence in a naive and immunosuppressed A/J mouse model of disseminated candidiasis. A/J mice, female 8 weeks, received a 200 mg/kg dose of cyclophosphamide (CY) by i.p on day -3. Prolonged neutropenia was maintained by giving each animal a 150mg/kg dose of CY i.p. every 7 days after infection. On day 0, mice were infected intravenously with $1x10^8$ or $2x10^8$ viable C. auris cells in 0.1 ml DPBS. As controls, age- and sex-matched A/J mice without CY treatment were challenged with the same two inoculum sizes of *C. auris* cells at the same time. **A.** Both naïve and immunosuppressed A/J mice are susceptible to *C. auris.* After intravenous challenge the inoculum of organisms ($1x10^8$), 100% mortality was observed in naïve A/J mice within 8 days even without CY pretreatment. **B & C**. Influence of immunosuppression on fungal burdens in brain, heart and kidney in mouse models of systemic C. auris infection was evaluated. A/J mice, with and without CY pre-treatment before *C. auris* 0386 intravenous challenge ($2x10^8$) were compared for fungal burdens in targeted organs, including heart **(B)** and kidney **(C).** Data are expressed as mean + S.D (n=5).

b. **Passive transfer of two mAb cocktails, significantly improved survival and decreased fungal burden in immunosuppressed BALB/c mouse model of invasive *C. tropicalis* and in immunosuppressed A/J model of invasive *C. glabrata* infection.**

We have identified two combination of mAbs (2C9+6E3 and 5A9+10E7) demonstrated to be more effective in protecting against disseminated candidiasis in our established immunocompromised mouse model of disseminated candidiasis caused by *C. tropicalis* (Figure 8) and *C. glabrata* (Figure 9), as compared to single mAb treatment.

By use of established BALB/c mouse model of disseminated *C. tropicalis* infection (Table 3), we combined two protective mAbs, including UP1 peptide specific mAb IgG3 5A9 and UP3 peptide specific mAb IgG1 10E7, into one cocktail with 1:1 ratio. We evaluated the mAb combination for protective efficacy, side by side with each single protective mAb given alone with the same dose. Survival data indicate that while the use of individual mAb is promising as a single prophylactic component, combination of two mAbs manifested the best protective efficacies (Fig. 8A).

Consistently, the group treated with double-mAb cocktail (5A9+10E7) had the least detectable CFUs in kidneys as compared to single mAb treated group (Fig. 8B). We concluded that combination of two protective mAbs provides the best protection in immunocompromised BALB/c mouse model of disseminated candidiasis by *C. tropicalis.*

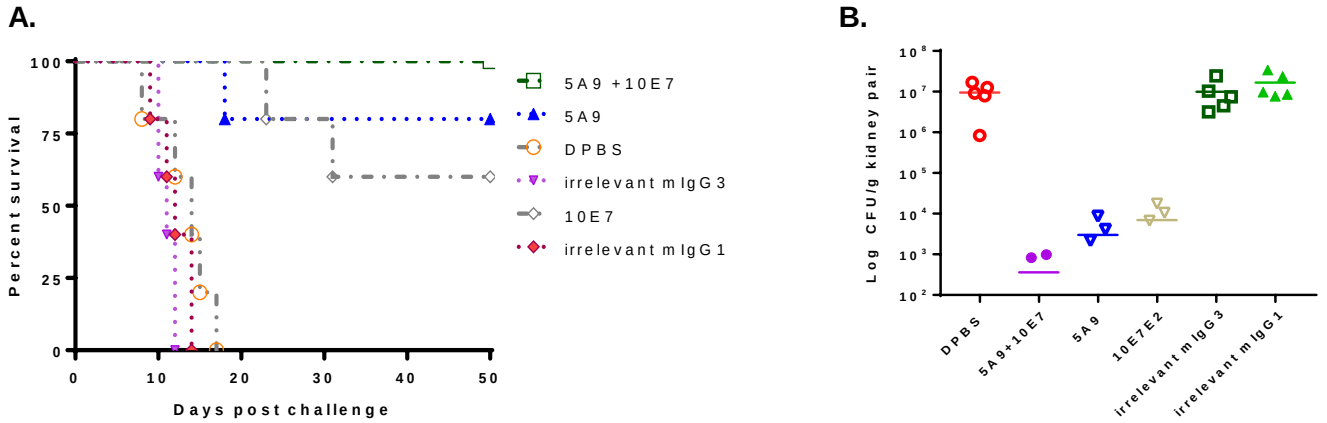


Fig. 8 MAbs 5A9 and 10E7 in combination conferred the best protection against *C. tropicalis* disseminated candidiasis in passive transfer experiments. Protective monoclonal antibodies (mAb IgG3 5A9 and mAb IgG1 10E7), specific for UP1 and UP3 peptide respectively, were obtained in my lab by use of standard hybridoma techniques. (A) BALB/c mice were each given by i.p. one dose of mAb 5A9 (100µg in 0.5ml DPBS), or mAb 10E7 (100µg in 0.5ml DPBS) or the mAbs in combination (100µg of each mAbs in 0.5ml DPBS) 4 hours before hematogensous challenge with a lethal dose of *C. tropicalis (*ATCC200956) cells (1x10^7). Mice received one dose of either mAb 5A9 or 10E7 had 60%-80% survival rate with prolonged survival as compared to control animals that received either DPBS or irrelevant mAbs; The mAbs combination (5A9+10E7) treatment was able to provide the best protection, 100% survival up to 50 days post-infection when experiment was terminated. (B) Each mAb treated group had reduced or non-detectable CFUs in their kidneys as compared to controls (p<0.001). Only two mice/survivors in 5A9+10E7 group had detectable lowest CFU in kidneys.

With the similar approach, use of established C5 deficient A/J mouse model of disseminated *C. glabrata* infection (Table 3), we combined two protective mAbs, including UP1 peptide specific mAb IgG2a 2C9 and UP2 peptide specific mAb IgG1 6E3, into one cocktail with 1:1 ratio. We evaluated the mAb combination for protective efficacy, side by side with each protective mAb given alone with the same dose. Survival data indicate that combination of two mAbs manifested the enhanced protective efficacies as compared to each single mAb treatment (Fig. 9A). Consistently, the group treated with the mAb cocktail (2C9+6E3) had the least detectable CFUs in kidneys as compared to single mAb treated group (Fig. 9B).

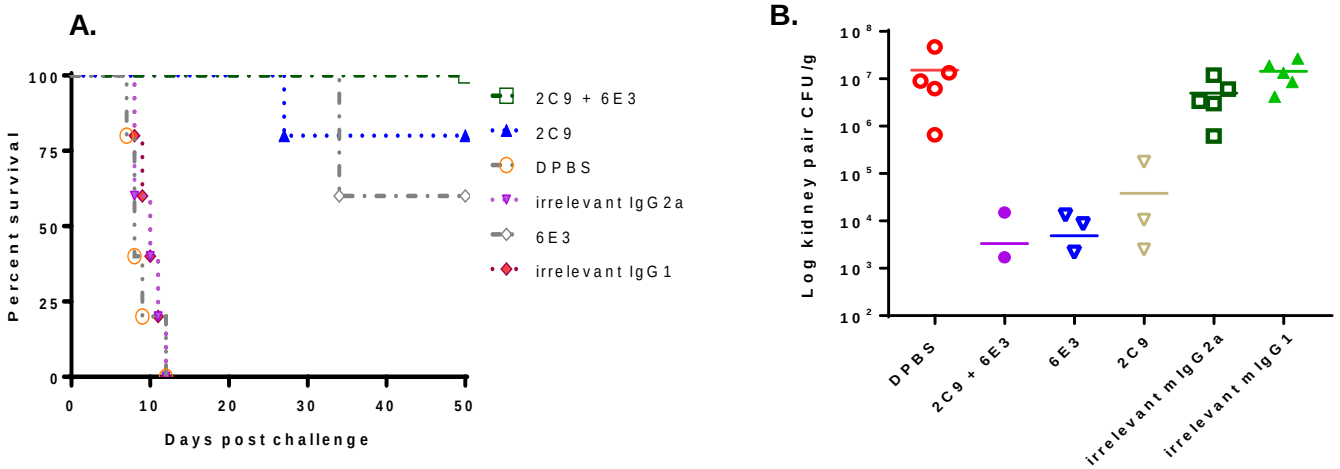


Figure. 9 MAbs 2C9 and 6E3 in combination conferred the enhanced protection against *C. glabrata* disseminated candidiasis in passive transfer experiments. Protective monoclonal antibodies mAb IgG2a 2C9 and mAb IgG1 6E3, specific for UP1 and UP3 peptide respectively, were obtained in my lab by use of standard hybridoma techniques. (A) A/J (C5 deficiency) mice were each given by i.p. one dose of mAb 2C9 (100µg in 0.5ml DPBS), or mAb 6E3 (100µg in 0.5ml DPBS) or the mAbs in combination (100µg of each mAbs in 0.5ml DPBS) 4 hours before hematogensous challenge with a lethal dose of *C. glabrata (*ATCC200918) cells ($1x10^8$). Mice received one dose of either mAb 2C9 or 6E3 had 60% or 80% survival rate with prolonged survival as compared to control animals that received either DPBS or irrelevant mAbs. The mAbs combination (2C9+6E3) treatment was able to provide the enhanced protection, 100% survival up to 50 days post-infection when experiment was terminated. (B) Each mAb treated group had significantly reduced or non-detectable CFUs in their kidneys as compared to controls (p<0.001). Only two mice in mAb combination group had detectable lowest CFU in kidneys as compared to single mAb groups.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We will complete all proposed studies in Aim 2 (we have achieved 70%), focusing on mAb-based therapy against multidrug-resistant *C. auris* invasive infection by use of established A/J intravenous mouse model. Proposed studies in Aim 3 will be accomplished during the next funding period, and the goal is to determine whether the mAbs have synergistic or enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis. We expect that new approaches to improve the efficacy of existing conventional antifungals will be developed.

1. *Candida auris* is a multi-drug resistant, health care-associated fungal pathogen, and has recently emerged as the first fungal pathogen to cause a global public health threat. Since there is no approved antifungal vaccine and current treatments are inadequate, antifungal antibodies could provide long-awaited novel therapies for use alone or in combination with antifungal agents. We will continue the work in Aim 2 to develop a mAb-based therapy target multiple antigenic epitopes to achieve the greatest efficacy aginst *C. auris* invasive infection.

2. After Aim 2 is accomplished 100%, each protective mAb will first be assessed in a murine model of disseminated candidiasis caused by *C. albicans* for its ability to enhance the efficacy of conventional antimicrobial drugs. First, we will focus on *C. albicans*, the most common disease-causing species (65%). Any mAb candidate that acts in concert with or augments protection when used with the drugs in combinational therapy will suggest a possibility of reducing the drug dose to a non-toxic level.

3. Furthermore, even *C. albicans* is the most common disease-causing species; the prevalence of disease caused by *non-albicans Candida* (*NAC*) species is on the rise, along with an increase in antifungal drug resistance. We will then focus on same three clinically significant *Candida* species

investigated in Aim 2. By the same experimental approaches, the combinational therapy of each mAb with conventional antifungal drug will be evaluated in established immunocompromised mouse models of disseminated candidiasis caused by *C. tropicalis* and *C. glabrata*, as well as in C5 deficient A/J mouse model of invasive *C. auris* infection (Table 3).

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Disseminated candidiasis in humans is the leading cause of hospital-related bloodstream infection in the US. Despite the availability of appropriate antifungal therapy, crude mortality in the last decade has remained high, ranging from 36 to 90%.

The goal of this proposal is to develop and establish preclinical proof-of-concept for the first universal therapeutic antibodies that protect against disseminated candidiasis caused by all the medically important *Candida* species. The principal discipline of the project will serve as the foundation for the future development of effective therapies for disseminated candidiasis. The milestones gained from the proposed research could be implemented in a dual-use capacity to benefit immunocompromised people of both civilian and military populations including, but not limited to, patients with severe burns, cancer, HIV, diabetes, neutropenia, leukemia, or those receiving immunosuppressive corticosteroids for bone marrow or organ transplantations. Based on the progress of therapeutic mAb cocktails, the new approach to further improve the efficacy of anti-fungal treatments by combining antibodies with existing conventional antifungal drugs for enhanced/synergy effects will be further accomplished. The milestones established in this proposal will be a significant leap forward in clinical management of invasive fungal infection. Antifungal antibodies could provide long-awaited novel therapies for use in combination with antifungal agents and may offer a safe, broad-spectrum prophylaxis for high-risk immunocompromised patients.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and / or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS

One manuscript was accepted by mSphere, in press (attached).

Full Title: Experimental mouse models of disseminated Candida auris infection

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Hong Xin, MD, Ph.D
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	Not applicable

Nearest person month worked:	3.6
Contribution to Project:	Dt. Xin has been responsible for the overall supervision and direction of the project. Conceive and direct all research work; analyze data; perform needed and critical bench work and animal challenge; establish immunocompromised mouse models to mimic clinical high-risk patient populations, guide and supervise the tests for efficacy of antibodies in mouse models; write manuscripts; present findings at scientific meetings; train personnel

Name:	Karen Eberle, B.S.
Project Role:	Research Associate III
Researcher Identifier (e.g. ORCID ID):	Not applicable
Nearest person month worked:	12
Contribution to Project:	Karen Eberle has been responsible for animal care, mAb preparation, *in vitro* culturing of *Candida*, *in vivo* passive transfer and challenge, and assessments of immunogenicity (ELISA) and fungal burden (CFUs in kidney). Responsible for the routine production and purification of mAbs, general lab work that support this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

None

8. SPECIAL REPORTING REQUIREMENTS

None.

9. APPENDICES

None.