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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 were evaluated and 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 have developed 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 100%
Subtask 1: Establish and maintain immunocompromised mouse models of invasive *NAC* infection	7-8	Achieved 100%
Subtask 2:Evaluating therapies of mAb cocktails against disseminated *NAC* infection	7-9	100
Subtask 3: Troubleshoot and adjust ratios of mAbs to achieve best efficacy.	10-12	100%
Milestone(s) Achieved:	12	100%
Milestone 2: Develop a broad-spectrum mAb therapies protect immunocompromised host against the medically important *Candida* species	12	Achieved 100%

Specific Aim 3		
Major Task 3: Determine whether the mAbs have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis.		Achieved 50%
Subtask 1: Test sub-therapeutic / full dose of mAb, FLC and AMB	13-15	Achieved 100%
Subtask 2: examine the combination therapy of each mAb/ mAb cocktails with fluconazole (FLC) and AMB	16-18	Achieved 60%
Milestone(s) Achieved:	18	
Milestone 3: Develop mAb therapies used as adjuncts to current antimicrobial therapy, to improve therapeutic efficacy in immunocompromised host		Achieved 40%

What was accomplished under these goals since the last annual report

Aim 3: Determine whether the mAbs have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis.

1) Major activities

New approaches to further improve the efficacy of mAbs by combining with existing conventional antifungal have been investigated. MAb is an attractive possibility to improve therapeutic outcomes for fungal infection, especially for combating *C. albicans*, as well as other medically important *Candida* species that are resistant to antibiotic therapy. Furthermore, the efficacy of therapeutic mAb can be augmented when used in combination with conventional antifungal therapy, or in the other way, the efficacy of antifungal therapy can be enhanced when used in combination with protective mAb, as it has been shown with Mycograb and amphotericin B (AMB) in patients with invasive candidiasis. The fact that specific antibodies are often synergistic with conventional antimicrobial therapy suggests that combination therapy with current antimicrobial regimens may confer potential advantages relative to either alone. In this Aim 3, each mAb identified in Aim 1 & 2 was assessed in murine disseminated candidiasis model for its ability to enhance the efficacy of conventional antimicrobial drugs.

2) Specific objectives

We first examined the combination therapy of each mAb with fluconazole (FLC), which has been considered as first-line antifungal medication for decades. By the same approach, we would then test each mAb combination therapy with conventional amphotericin B (AMB, also called Fungizone), which remains the standard therapy for invasive candidiasis, especially for multi-drug resistant

Candida species that are resistant to both fluconazole and an echinocandin. Although AMB resistance appears uncommon among isolates of *C. albicans, C. tropicalis,* and *C. parapsilosis*, there is a certain limit to use AMB because the antifungal drug causes a severe renal damage. We have successfully reported combination immunotherapy of mAb B6.1 with FLC or AMB augments therapeutic effect to disseminated candidiasis in previous studies; therefore, we can follow the same approach described as before.

All the mAbs listed at **Table 1** have been evaluated and demonstrated to provide enhance or synergy in the protection against disseminated candidiasis caused by *C. albicans* in immunocompetent mice (C57BL/6, BALB/c and A/J) when used as cocktails. In Aim 1 & 2, we have shown 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. In Aim 3, the identified protective mAbs were further investigated in animal models to determine whether the mAbs have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis. New approaches to further improve the efficacy of with existing conventional antifungal by combining mAbs have been developed and established here. Experimental design for tested groups (mAb + antifungal FLC) and control groups were listed in Table 1.

Table 2 is a concise summary for the experimental results we have obtained in the proposed study of Aim 3. Our data are important and promising since mAb is an attractive possibility to improve therapeutic outcomes for fungal infection, especially for combating *C. albicans* that are resistant to antibiotic therapy. As shown in Table 2, the efficacy of therapeutic mAb can be augmented when used in combination with conventional antifungal therapy, as it has been shown with Mycograb and amphotericin B (AMB) in patients with invasive candidiasis. The more important findings we have here is that the efficacy and effect of antifungal, for example FLC, can be significantly improved even with subtherapeutic dose level, which means much less toxicity especially for immunocompromised patients, who didn't respond well to antifungal treatment as immunocompetent people and are the highest risk group for invasive candidiasis. The fact that specific antibodies are often synergistic with conventional antimicrobial therapy suggests that combination therapy with current antimicrobial regimens may confer potential advantages relative to either alone. Our data have demonstrated here that the identified mAbs have great potential to be applied with conventional antifungal agent in human clinical situations. In this Aim, each mAb will be further assessed in murine disseminated candidiasis model for its ability to enhance the efficacy of the other conventional antimicrobial drugs, such as AMB, as proposed in Aim 3.

Descripting the results of Aim 3 in detail: Regard to survival data (Fig. 1-3), BALB/c mice were given an i.p dose of each mAb with or without FLC four hours after hematogenous challenged with a lethal dose of *C. albicans* SC5314 cells. Mice that received half effective dose of mAb combined with FLC subtherapeutic dose treatment had 60% survival for 2C9 + FLC (Fig. 2), 80% survival rate for 5A9 + FLC (Fig. 2), and 40% survival for 6E3 + FLC (Fig. 3) with significantly prolonged survival (all the survival experiments were terminated at day 60 post-challenge) as compared to groups received FLC subtherapeutic or full dose treatment ($p<0.01$). All the DPBS control animals succumb to the invasive infection within 5-10 days. In addition, surviving animals that received either the mAb alone treatment or mAb + FLC had significantly reduced or non-detectable fungal burdens in their kidneys and brains as compared to FLC controls (subtherapeutic and full therapeutic dose groups). Our data obtained here provided strong additional evidence for the protection being due to the synergy with protective mAb. Statistical evaluations were performed using GraphPad Prism 7.0

(GraphPad). *P* values were calculated using a two-tailed Mann-Whitney test (data were considered significant when *P* values were below 0.05).

Table 1. Experimental design of testing efficacy of mAb combined antifungal treatment.

Therapeutic mAb + FLC **Both with subtherapeutic dose**	**Control #1 (mAb)** **Half effective dose**	**control #2 (FLC)** **subtherapeutic dose**	**control #3 (FLC)** **minimal effective dose**
2C9 + FLC (Done)	2C9	FLU 0.8mg/kg	FLC 1.6mg/kg
5A9 + FLC (Done)	5A9	FLU 0.8mg/kg	FLC 1.6mg/kg
6E3 + FLC (Done)	2C9	FLU 0.8mg/kg	FLC 1.6mg/kg
10E7 + FLC	10E7	FLU 0.8mg/kg	FLC 1.6mg/kg
5A1 + FLC	5A1	FLU 0.8mg/kg	FLC 1.6mg/kg
1D5 + FLC	1D5	FLU 0.8mg/kg	FLC 1.6mg/kg
7A3 + FLC	7A3	FLU 0.8mg/kg	FLC 1.6mg/kg

Shaded area shows the work has been accomplished

3) Significant results and key outcomes

Several combinations of mAb + FLC (2C9 + FLC, 5A9 + FLC and 6E3 +FLC) were demonstrated to be more effective in protecting against disseminated candidiasis by C. *albicans* (SC5413) as compared to FLC alone (both subtherapeutic dose and full effective dose) in BALB/c mouse model of disseminated candidiasis (Table 2). The protection was evidenced by significantly increased survival rate by day 60 post-infection and reduced or non-detectable fungal burden (Colony forming units, CFUs) in kidney and brain. All three mAbs + FLC combination showed an enhanced / synergistic effect as compared to antifungal treatment alone, which represented a critical improvement in antifungal therapy. With this supporting data, therapeutic combinations that include mAb and antifungal in the form of different isotypes and epitope specificities may be further designed, since combination of functional mAb and FLC could provide more protection and at the same time decrease toxicity of common antifungals as compared to conventional antifungal treatment by itself.

Experiments with obtained results: We first examined the combination therapy of each mAb with fluconazole (FLC), which has been considered as first-line antifungal medication for decades. By the same approach, in the next 4-5 month, we will test each mAb combination therapy with conventional amphotericin B (AMB, also called Fungizone), which remains the standard therapy for invasive candidiasis, especially for multi-drug resistant *Candida* that are resistant to both fluconazole and an echinocandin. We have successfully reported combination immunotherapy of each mAb 5A9, 2C9 and 6E3 with FLC augments therapeutic effect to disseminated candidiasis; therefore, we will follow the same approach to evaluate each mAb combination therapy with conventional amphotericin B (AMB).

- **Synergistic / enhanced efficacy of mAb combined antifungal therapy on survival and fungal burdens in mouse model of disseminated candidiasis.**

Briefly, subtherapeutic / full dose of FLC combined with subtherapeutic dose of each mAb (half dose of effective dose) was administered 4 hours post-infection, survival data were shown in Figure 1-3. All tested mAbs, 2C9 (Fig. 1), 5A9 (Fig. 2) and 6E3 (Fig. 3), each synergized with FLC (subtherapeutic dose) to enable mice to survive lethal challenge with significantly prolonged survival time ($p<0.01$), as compared to FLC treatment alone, regardless of full therapeutic dose or subtherapeutic dose. For mAbs 2C9 and 5A9, we do see the synergy of mAb + FLC treatment as compared to single mAb treatment alone, however, for mAb 6E3, there is no significant difference in survival and fungal burden between the groups received 6E3 + FLC and treated with mAb 6E3 alone (Fig. 3). However, for all three tested mAbs, each was able to enhance / synergize with FLC as compared to FLC alone, which showed a new approach to further improving the efficacy of antifungal by combining with protective mAbs.

In order to further examine the effects of the combination therapy on fungal load in targeted organs, parallel experiments for organ CFU analysis were done at 4 different time points when treatment was given. Briefly, treatments were kept the same as that in survival experiments, subtherapeutic dose of FLC combined with subtherapeutic dose of each mAb (half dose of effective dose) was administered 1, 4, 8,12 hours post-infection. Group treated with either subtherapeutic or full dose of FLC, or each mAb alone was used as control. Group receiving DPBS buffer only was used as negative control. All experimental groups were sacrificed at 48 hours poste-infection and CFUs in both kidney pair and brain were analyzed.

Although mice given the subtherapeutic dose of FLC (0.8 mg drug/kg body weight) were as susceptible as the animals that received DPBS buffer, each mAb plus FLC at 0.8 mg/kg dose showed much more efficient therapeutic efficacy as compared to FLC at 0.8 mg/kg alone and even at 1.6 mg/kg alone. We found the augmentation response was related to each specific mAb: 1), FLC 0.8mg/kg combined with Isotype control mAb could not provide any enhanced protection as compared to either FLC or mAb isotype control treatment (data not shown). 2), Each of tested mAbs when combined with FLC was able to provide enhanced protective efficacy against disseminated candidiasis as compared to of FLC treatment alone. 3), For mAbs 2C9 and 5A9, each mAb and FLC combination can also provide enhanced protection as compared to mAb treatment alone, however, there is no difference in both survival and organ fungal burden between groups treated with mAb 6E3 alone and treated with mAb 6E3 + FLC, which showed mAb 6E3 couldn't synergize with FLC to increase efficacy of 6E3 solo treatment, but 6E3 + FLC did improved efficacy of FLC treatment alone (Fig. 3). Consistently differences in kidney CFU and survival times between the combination therapy groups and the FLC alone controls are statistically significant ($p<0.01$). We plan to further test FLC at 0.4 mg/kg to determine if we can further lower the FLC dose to achieve the same therapeutic efficacy. We will then test the efficacy of FLC and mAb combination therapy given 24h and 48h post-infection. Since antibody therapy was generally effective only early in the course of infection while antibiotic therapy maintained efficacy even when given late in the course of the infection, we will further test if mAb can complement / synergize the efficacy of FLC treatment to the disease when given at later time points. The same experimental approaches of mAb with FLC will be performed with AMB. Any mAb candidate acts in concert with FLC/AMB in combination therapy that augments protection will suggests a possibility of reducing FLC/AMB dose to non-toxic level. For future study, we will further determine if the mAb(s) identified in this Aim have synergy/enhanced efficacy when combined with conventional antimicrobial drugs against disseminated candidiasis caused by multiple *Candida* species.

Table 2. Combined universal-peptide–specific with antifungal drug FLC conferred synergy / enhanced protection against systemic candidiasis as compared to FLC alone treatment.

Survival by day 60 post infection				
Protective mAb cocktails	**Control #1**	**control #2**	**Protection of mAb + FLC combination**	**Synergy of combination**
2C9 + FLC	2C9 (40%)	FLC (0%)	60%	**Yes to 2C9/FLC**
5A9 + FLC	5A9 (60%)	FLC (0%)	80%	**Yes to 5A9/FLC**
6E3 + FLC	6E3 (40%)	FLC (0%)	40%	Yes, to FLC alone

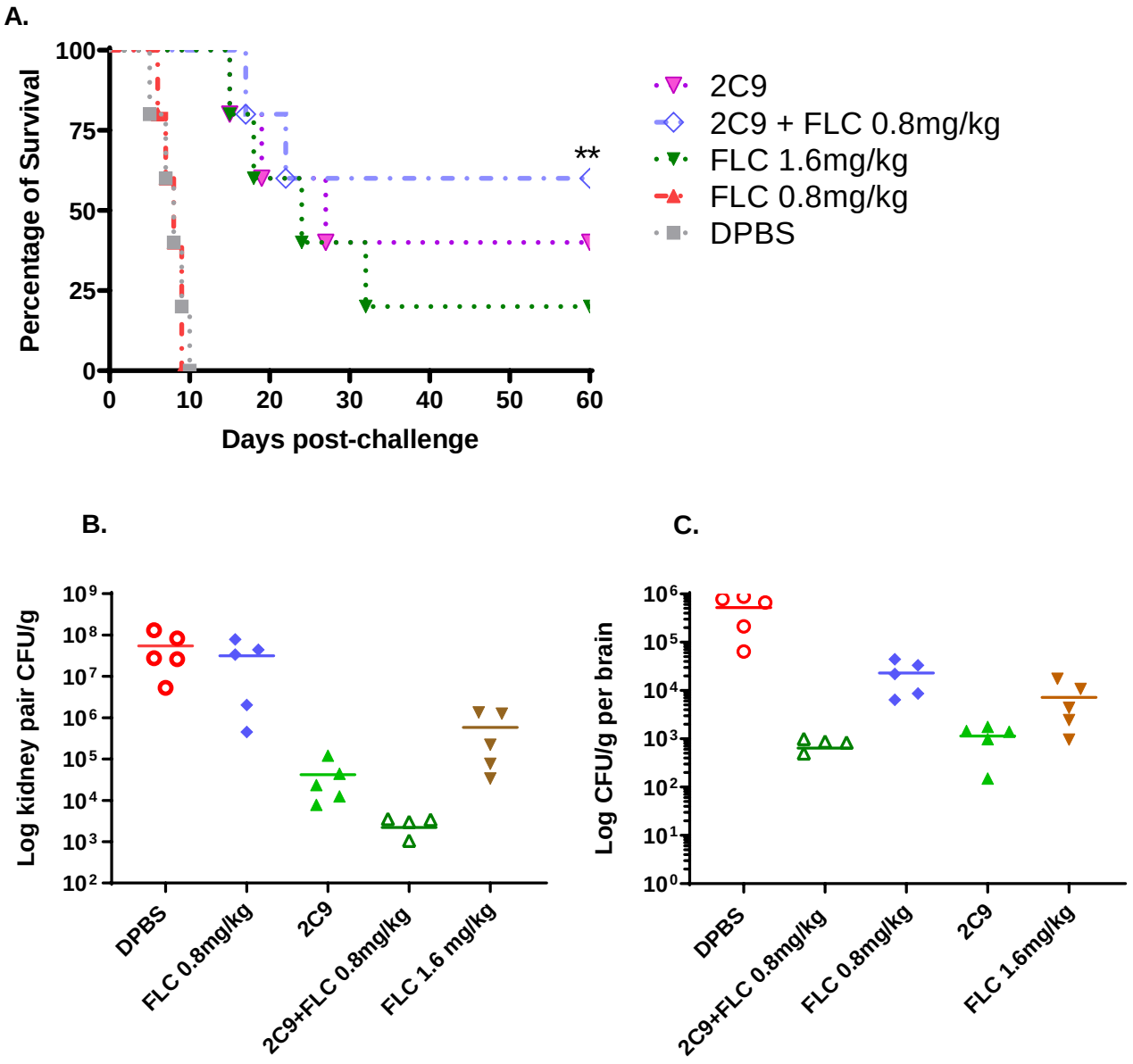


Figure 1. Effect of mAb 2C9 and FLC combination treatment on the survival of mice and CFU in targeted organs in BALB/c mouse model of disseminated candidiasis. For each mAb + FLC survival experiment, all groups of mice were challenged with lethal dose of *C. albicans* yeast cells (*C. albicans* SC5314, 5x10e5 in 100ul DPBS), 4 hours later, each of 5 groups of mice were treated with DPBS, FLC 0.8mg/kg, FLC 1.6mg/kg, mAb 2C9 half dose of effective dose, 2C9 + FLC 0.8mg/kg. Survival experiments were terminated at day 60 post-challenge and CFU from targeted organs, kidney and brain, were analyzed as before. (**A**) BALB/c mice

of all groups were monitored for survival after challenge with lethal dose of *C. albicans* yeast cells. The group receiving treatment of 2C9 and FLC in combination had 60% survival. The group treated with 2C9 alone have 40% survival. Both 2C9 alone and 2C9+FLC groups survive significantly longer than the both groups receiving FLC treatment, either with subtherapeutic dose or full therapeutic dose. Consistently, the group treated with 2C9 + FLC had the least CFUs in both kidney (**B**) and brain (**C**) among all the groups ($p<0.01$). One out of five mice in 2C9 + FLC treated group had non-detectable CFU in kidney and brain, indicating some survivors were able to clear up the fungal burden from the targeted organs.

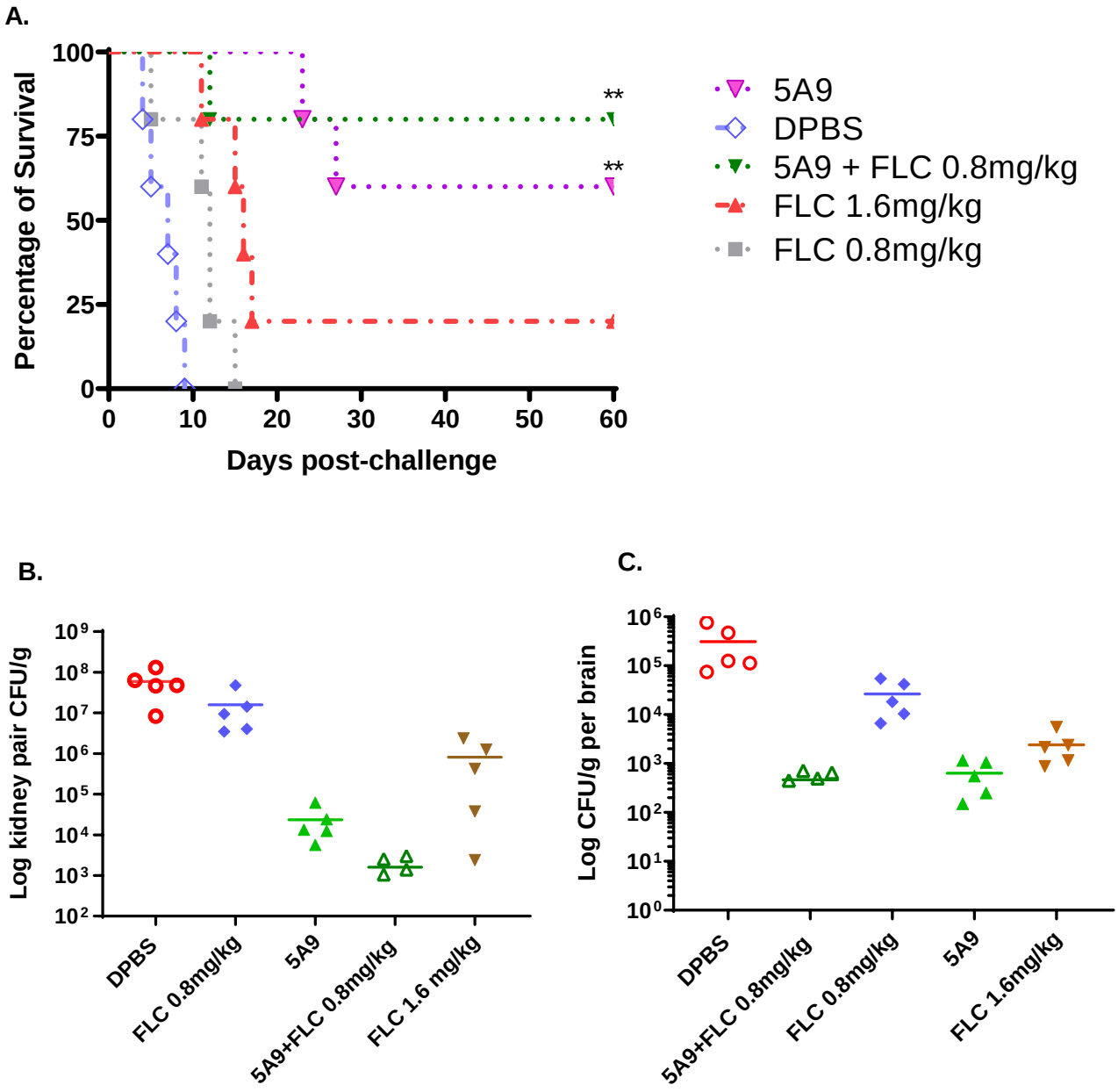


Figure 2. Effect of mAb 5A9 and FLC combination treatment on the survival of mice and CFU in targeted organs in BALB/c mouse model of disseminated candidiasis. All groups of mice were challenged with lethal dose of *C. albicans* yeast cells (*C. albicans SC5314*, 5x10e5 in 100ul DPBS), 4 hours later, each group of mice were treated with DPBS, FLC 0.8mg/kg, FLC 1.6mg/kg, mAb 5A9 half dose of effective dose, 5A9 + FLC 0.8mg/kg. Survival experiments were terminated at day 60 post-challenge and CFU from targeted organs, kidney and brain, were analyzed as before. (**A)** BALB/c mice of all groups were monitored for survival after challenge with lethal dose of *C. albicans* yeast cells. The group receiving treatment of 5A9 and 5C9 + FLC in

combination had 80% survival by day 60 post-infection. The group treated with 5A9 alone have 60% survival. Both 5A9 alone and 5A9 + FLC groups survived significantly longer than the either FLC treated group with subtherapeutic dose or full therapeutic dose. Consistently, the group treated with either 5A9 or 5A9 + FLC had the significantly decreased CFUs in both kidney (**B**) and brain (**C**) as compared to FLC groups ($p<0.01$**).**

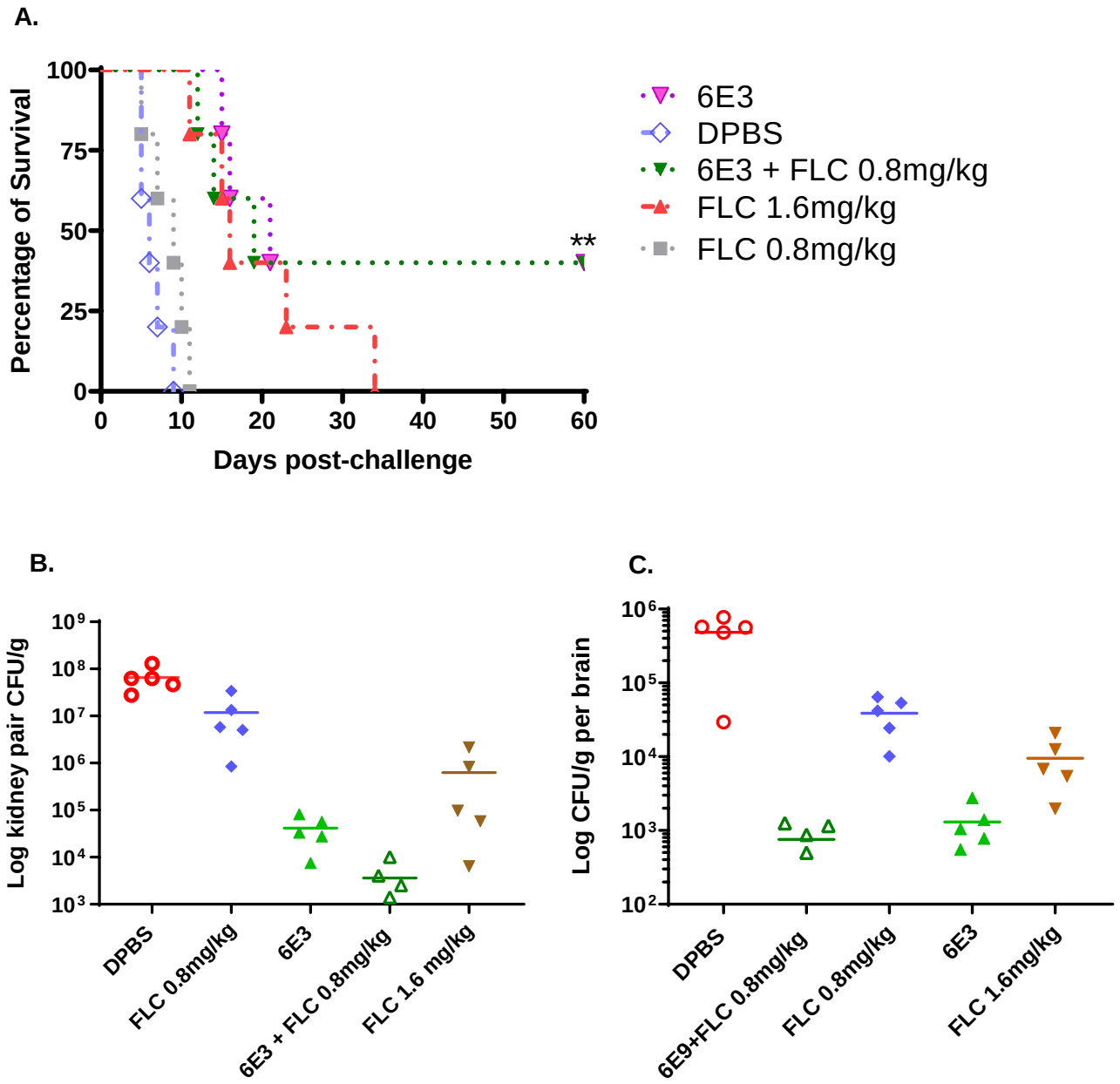


Figure 3. Effect of mAb 6E3 and FLC combination treatment on the survival of mice and CFU in targeted organs in BALB/c mouse model of disseminated candidiasis. For groups of mice were challenged with lethal dose of *Candida* yeast cells (C. *albicans* SC5314, 5x10e5 in 100ul DPBS), 4 hours later, each of 5 groups of mice were treated with DPBS, FLC 0.8mg/kg, FLC 1.6mg/kg, mAb 6E3 half dose of effective dose, 6E3 + FLC 0.8mg/kg. Survival experiments were terminated at day 60 post-challenge and CFU from targeted organs, kidney and brain, were analyzed as before. **(A)** BALB/c mice were monitored for survival after challenge with lethal dose of C. *albicans* yeast cells. The group receiving treatment of 6E3 and FLC in combination had 40% survival. The group treated with 6E3 alone have 40% survival too. Both 6E3 alone and 6E3 + FLC groups survive significantly longer than the both FLC treated groups ($p<0.01$) with subtherapeutic dose and full

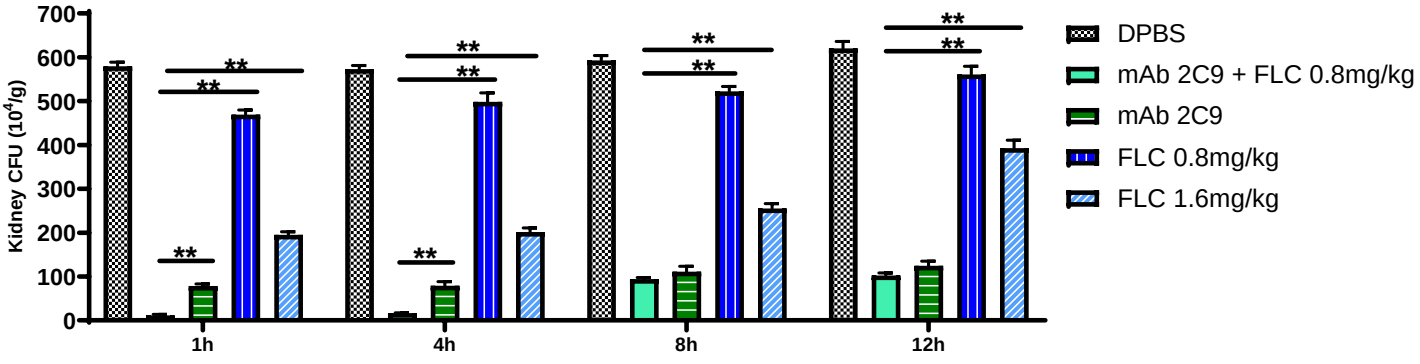
therapeutic dose. Consistently, the group treated with 6E3 + FLC had the least CFUs in kidney **(B)** and brain **(C)** among all the groups ($p<0.01$).

- **Synergistic / enhanced efficacy of mAb combined antifungal therapy, given at different time points, on fungal burdens in mouse model of disseminated candidiasis at different time points**

To examine the effects of the combination of mAb and antifungal therapy on fungal burdens in targeted organs at different time points given after challenge, parallel experiments for organ CFU analysis were done to compare efficacy of treatment given at four different time points post-infection. Briefly, treatments were kept the same as that in survival experiments (Fig. 1-3), after challenge, subtherapeutic dose of FLC combined with subtherapeutic dose of each mAb (half dose of effective dose) was administered at 1, 4, 8,12 hours post-infection. Group treated with either subtherapeutic or full dose of FLC, or each mAb alone was used as control. All experimental groups were sacrificed at 48 hours poste-infection and CFUs in both kidney pair and brain were analyzed.

Although mice given the subtherapeutic dose of FLC (0.8 mg drug/kg body weight) were as susceptible as the animals that received DPBS buffer, CFU results in kidney demonstrated that each mAb combined with FLC at 0.8 mg/kg dose showed much more efficient therapeutic efficacy as compared to FLC treatment at 0.8 mg/kg alone and even at therapeutic dose of FLC1.6 mg/kg alone. Consistently with survival data, greatly decreased CFUs in kidney between the mAb + FLC combination therapy groups and the FLC alone controls are statistically significant ($p<0.01$). Since antibody therapy was generally effective only early in the course of infection while antibiotic therapy maintained efficacy even when given late in the course of the infection, our results demonstrated mAb can complement / synergize the efficacy of FLC treatment to the disease when given by 12 hours post-infection.

A.



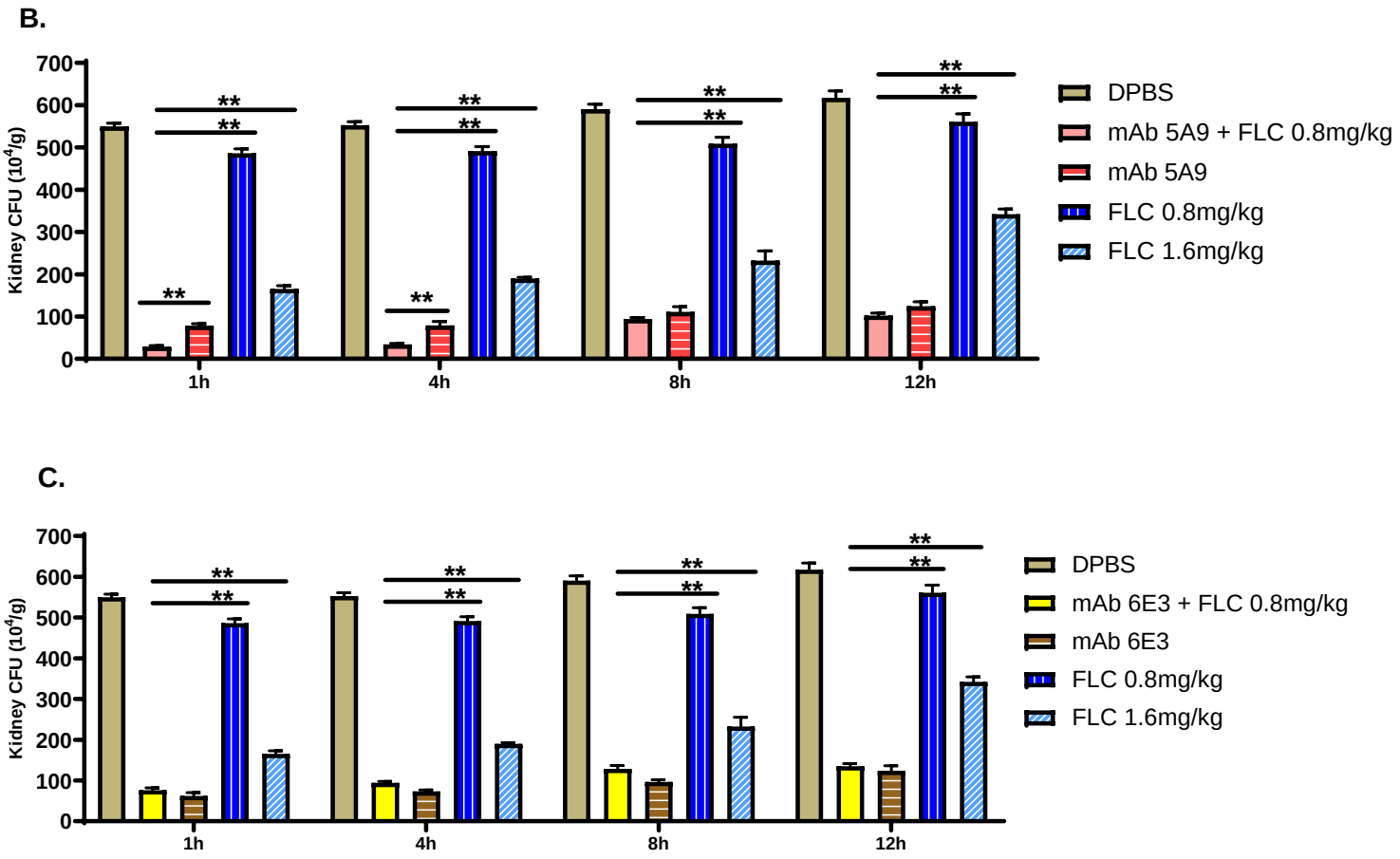


Figure 4. All the mAbs (IgG2a 2C9, IgG3 5A9, IgG1 6E3) have synergized therapeutic potential when combined with FLC against disseminated candidiasis if given by 12h post-infection. BALB/c mice (7 weeks, female) were infected i.v. with lethal dose 5x10^5 viable *C. albicans* yeast cells (SC5314); at 1, 4, 8, 12h post-infection, they received the pre-determined half dose of each protective mAb, or mAb combined with a pre-determined subtherapeutic dose of FLC 0.8mg/kg, or full therapeutic dose FLC 1.6mg/kg. The group received DPBS buffer was used as negative control. Forty-eight hours after the infection, the animals of all experimental groups were sacrificed, and the resulting *C. albicans* colony forming units (CFU) from kidney homogenates was analyzed and shown as an indicator of disease severity. Resistance to disseminated candidiasis was assessed by determining *C. albicans* CFU in the kidney pair of each mouse in different groups. **(A)** *C. albicans*-infected mice treated with mAb 2C9 and FLC combination given at each time point post-infection all had significant reduction in fungal CFUs compared to mice receiving FLC (0.8mg/kg or 1.6mg/kg) alone at the same time point ($p<0.01$). When 2C9 + FLC or 2C9 alone was given at 1 and 4 hours post-infection, CFU in kidney of the group treated with 2C9 + FLC was significantly decreased as compared to that of the group treated with 2C9 alone, indicating 2C9 combined with FLC also provided enhanced protection as compared to 2C9 alone. **(B)** Similarly, administration of mAb 5C9 with FLC given i.p. at 1, 4, 8, 12h post-infection had enhanced / synergized therapeutic efficacy as compared to FLC treatment alone, evidenced by significantly reduced CFUs in kidney as compared to FLC alone treatment at each time point. Furthermore, administration of mAb 5A9 combined with FLC given at 1- and 4-hours post-infection also obtained enhanced therapeutic potential as compared to single mAb 5A9 treatment, evidenced by significantly reduced CFU in kidney as compared to mAb 5C9 alone treatment at each time point. **(C)** CFU in kidney of groups treated with single mAb 6E3 or 6E3 + FLC was significantly reduced ($P<0.01$) as compared to that in FLC treated mice at the same time points. However, 6E3 combined FLC treatment provided the comparable protection as 6E3 alone treatment according to CFU in kidney of both groups. The consistent observation for all mAbs is, each mAb combined with FLC can provide synergized / enhance therapeutic efficacy as compared to FLC treatment alone.

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 3 (we have achieved at least 50%), focusing on mAb-antifungal based therapy against other medically important *Candida* species, for example C. *glabrata*, and C. *tropicalis*, as well as including multidrug-resistant *C. auris* invasive infection by use of established A/J intravenous mouse model. We are the first lab the established naïve A/J mouse model of disseminated candidiasis without any immunosuppressant interference. Animal experiments and work progress have been affected badly by COVID-19 pandemic due to lockdown, stay at home situations and shorthand at animal facility. Now LSUHSC requested work remotely at home under second peak of COVID-19 positive increase. Lab people and PI have been striving to ensure the proposed studies in Aim 3 be accomplished by the end of 2020, and the goal is to determine and validate the synergistic or enhanced efficacy when mAb(s) being combined with conventional antimicrobial drugs against disseminated candidiasis. We expect that the new approaches established in this study can greatly improve the efficacy of existing conventional antifungals in human clinical situations.

Plans in detail: After Aim 3 is accomplished with evaluating and validating each mAb in table 1 combined with FLC, each protective mAb will be further assessed in a murine model of disseminated candidiasis caused by *C. albicans* for its ability to enhance the efficacy of anther conventional antimicrobial drug, amphotericin B (AMB). First, we have been focusing 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. 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. We will test the same combination of identified (mAb + antifungal) in mouse models of disseminated candidiasis caused by other medically important species, including C. auris.

1. **IMPACT**

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

The impact keeps the same. With more progress we obtain from Aim 3 in this study, we are developing and validating a more efficiency and less toxic strategy in treating disseminated candidiasis, especially in the immunocompromised populations, and the disease caused by drug resistant *Candida* species. 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.

2. 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.

3. PRODUCTS

Nothing to Report.

4. 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, and animal experiments, 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

5. SPECIAL REPORTING REQUIREMENTS

None.

6. APPENDICES

None.