A Cross-kingdom Vaccine Protects against Multiple Healthcare-associated Infections

Poster #97

Shakti Singh[1,2], Ashley Barbarino[1], Eman Youssef[1,3], Sunna Nabeela, Teklegiorgis Ghebremariam[1], Sondus Alkhazraji[1], Ashraf S. Ibrahim[1, 2]

[1]The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA. [2]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA. [3]Biotechnology Department, Beni-Suef University, Egypt

Email: Ibrahim@Lundquist.org
shakti.singh@Lundquist.org

FUNDING: NIAID R01AI141202

INTRODUCTION

- Infections due to multidrug resistant (MDR) pathogens have been on the rise.
- The pharmaceutical drug industry investment in anti-infective has not kept with the MDR infection rising pace.
- Alternative strategies to combat infections due to MDR pathogens are needed.
- Novel vaccine/immunotherapeutic strategies to prevent and/or treat MDR pathogens would immensely benefit global health.
- The *Candida albicans* (*CA*) NDV-3A vaccine (Als3p + alum) protects in preclinical models of *CA* and MDR *C. auris* infections due to the presence of Als3p orthologs.
- NDV-3A also protected women from recurrent-vulvovaginal candidiasis.
- The *CA*, Hyr1 antigen also protects against CA infections in preclinical models.
- Hyr1p shares structural homology with hemagglutinin protein (FhaB) and the outer membrane protein A (OmpA) conserved in MDR Gram-negative bacteria (GNB) and protects mice from pneumonia due to *Acinetobacter baumannii* (*AB*), *Klebsiella pneumoniae* (*KP*), and *Pseudomonas aeruginosa* (*PA*).

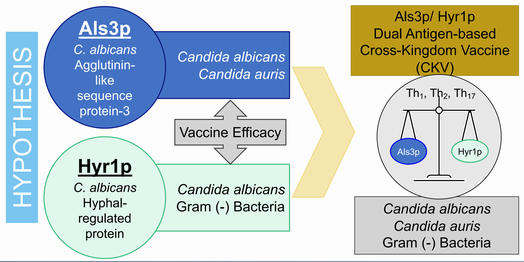

Table 1: CAF01™ Adjuvant (Statens Serum Institute, Denmark) formulated dual antigen –based vaccine

#	Formulation	Als3p/ Dose	Hyr1p/ Dose
1	0/0	0 ug	0 ug
2	10/10	10 ug	10 ug
4	30/10	30 ug	10 ug
5	30/3	30 ug	3 ug

METHODS

- For immunogenicity evaluation, the 4-6 weeks old outbred CD-1 mice were immunized on day 0 and 21 subcutaneously (SC). Two weeks post boost, specific antigen antibody titers and T cell immune responses (IFN-γ [Th1], IL-4 [Th2] and IL-17 [Th17]) were determined using ELISA and Triple color FluroSpot assay kits (CTL Immunospot), respectively.
- For efficacy evaluation against *CA*, mice were vaccinated on day 0, 21, and 35 and infected on day 49 intravenously with $2x10^5$ cells/ mouse.
- For efficacy evaluation against *C. auris* and GNB, mice were vaccinated on day 0 and 21, followed by infection on day 35. The mice were immunosuppressed by administration of cyclophosphamide and cortisone acetate on day -2 and +3, relative to infection. For *C. auris*, mice were infected through intravenous injection with $5x10^7$ cells/ mouse. For GNB infection, mice were infected through inhalation (*AB*) or intratracheal instillation *(KP or PA)*.
- Survival by Day 21 post infection served as a primary endpoint, while tissue pathogen burden of target organs by Day 4 post infection served as a secondary endpoint.

REFERENCES

1. Luo G, Ibrahim AS, French SW, Edwards JE Jr., Fu Y. Active and passive immunization with Rhyr1p-n protects mice against hematogenously disseminated candidiasis. PLoS One (2011) 6:e25909.
2. Spellberg BJ, Ibrahim AS, Avanesian V, Fu Y, Myers C, Phan QT, et al. Efficacy of the anti-candida Ralς3p-n or Ralς1p-n vaccines against disseminated and mucosal candidiasis. J Infect Dis (2006) 194:256–60.
3. Ibrahim AS. The anti-candida vaccine based on the recombinant n-terminal domain of Als1p is broadly active against disseminated candidiasis. Arch Microbiol (2006) 74:3039–41.
4. Luo G, Ibrahim AS, Spellberg B, Nobile CJ, Mitchell AP, Fu Y, et al. Candida albicans Hyr1p confers resistance to neutrophil killing and is a potential vaccine target. J Infect Dis (2010) 201:1718–28.
5. Uppuluri P, Singh S, Alqarihi A, Schmidt CS, Hennessey JP Jr, Yeaman MR, et al. Human anti-Als3p antibodies are surrogate markers of NDV-3A vaccine efficacy against recurrent vulvovaginal candidiasis. Front Immunol (2018) 9:1349.
6. Singh S, Uppuluri P, Mamouei Z, Alqarihi A, Elhassan H, French S, Lockhart SR, Chiller T,, et al. The NDV-3A vaccine protects mice from multidrug-resistant Candida auris infection. PLoS Pathogens. 2019 Aug 5; 15(8): e1007460

RESULTS

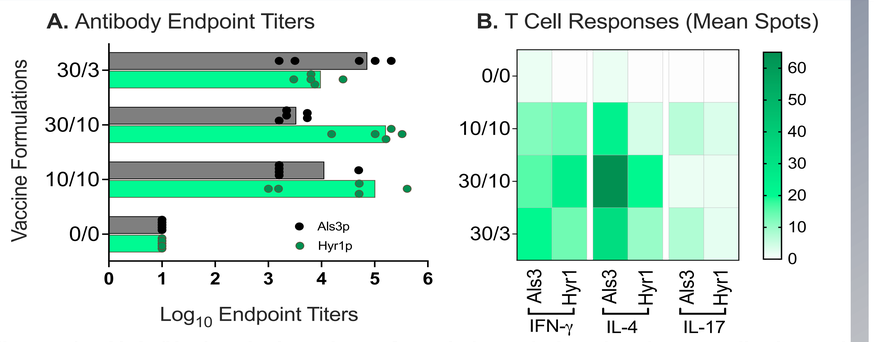

Figure 1. Als3p/Hyr1p dual antigen formulations induced robust antibody and Th1/Th2/Th17 cells responses against both antigens. 4-6 weeks old CD-1 mice (N= 5/group) were vaccinated sub-cutaneously on day 0 and 21. Two weeks after final vaccination, serum IgG titers and splenocyte T cell responses were evaluated using ELISA and FluroSpot assay, respectively.

Infection Models	Als3p/ Hyr1p (in ug) +CAF01											
	0/0 (Placebo)	30/10				10/10			30/3			
	% Survival efficacy | Median Survival Time in Days (p value)											
C. albicans	0%	12	40%	17	(0.0009)	40%	16	(<0.0001)	45%	18	(0.0001)
C. auris	0%	10	30%	14	(0.0016)	50%	19	(<0.0001)	55%	21	(<0.0001)
A. baumannii	5%	7	40%	14	(0.0174)	45%	14	(<0.0001)	20%	8	(0.181)
K. pneumoniae	15%	14	60%	21	(0.0029)	50%	19	(0.017)	50%	21	(0.0054)
P. aeruginosa	0%	2	10%	5	(0.0154)	40%	5	(.0186)	0%	5	(.0311)

Table 2. Survival efficacy of Als3p/Hyr1p formulations against Candida species and GNB infections. Both 30/10 and 10/10 vaccine formulations protected significantly against all five infections. Specifically, for fungal infections, 30/10 and 10/10 formulations showed 30-40% and 40-50% survival efficacies (vs. 0% for placebo), respectively. The 30/10 and 10/10 formulations showed 40% and 45% survival (vs. 5% placebo) against *AB*, 60% and 50% survival (vs. 15% placebo) against *KP*, and 10% and 40% survival (vs. 0% placebo) against *PA*, respectively.

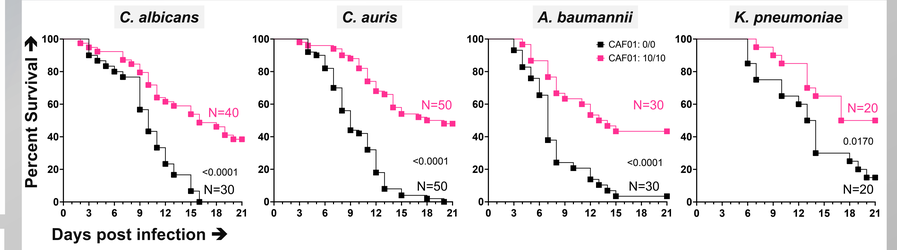

Figure 2. Dual antigen vaccine 10/10 protects against systemic candidiasis due to *C. albicans* and MDR *C. auris* and GNB pneumonia due to *A. baumannii* and *K. pneumoniae*. Two weeks after the booster immunization, CD-1 mice were infected with Candida species or Gram-negative bacteria (*AB* or *KP*). Mice survival were compared by Mantel-Cox test.

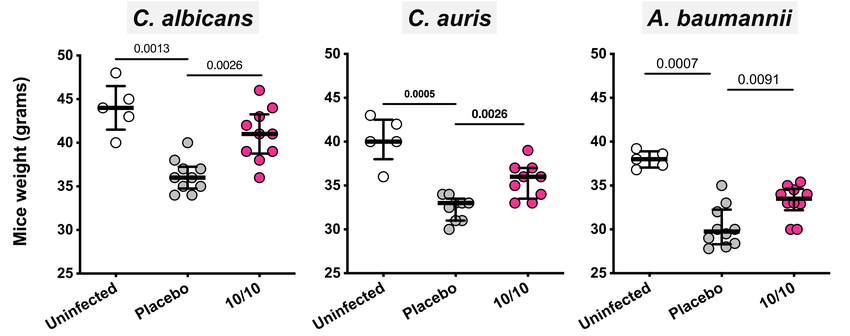

Figure 3. Dual antigen vaccine 10/10 prevents weight loss due to systemic candidiasis (*C. albicans, C. auris*) and *A. baumannii* pneumonia. CD-1 Mice (N=9-10/group) weights were compared after 4 days of infection by Mann-Whitney Test (Median ± IQR).

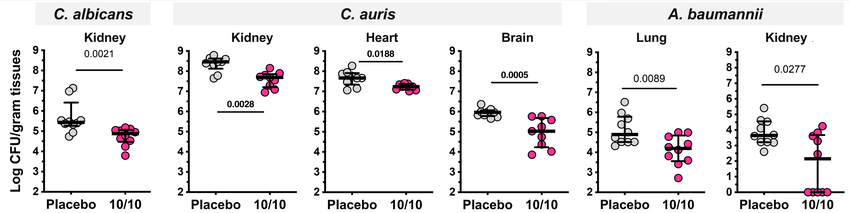

Figure 4. Dual antigen vaccine 10/10 reduced tissue microorganism burden in the target organs of mice infected with *C. albicans*, *C. auris* or *A. baumannii*. Two weeks after vaccination, CD-1 mice (N=9-10 /group) were infected with Candida spp. or *A. baumannii*. After 4 days of infection, tissue microbial burdens were determined and compared by Mann-Whitney test (Median ± IQR).

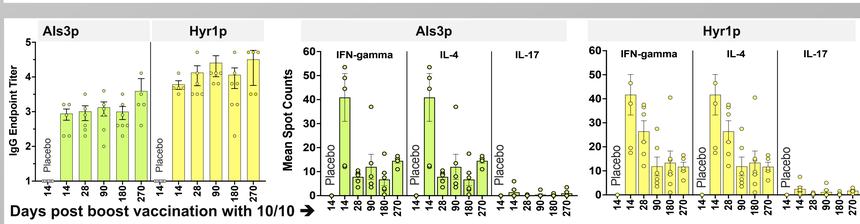

Figure 5. Dual antigen vaccine 10/10 induces durable immune responses against vaccine antigens Als3p and Hyr1p. Two weeks after vaccination (day 0, 21), vaccine antigen-specific immunity (IgG antibody and T cells producing IFN-gamma or IL-4 or IL-17) were monitored at different time points, using ELISA and FluroSpot assay. Data presented as mean ± SE of N=5 mice/group.

CONCLUSIONS

The Als3p/Hyr1p dual antigen vaccine induced a robust protective immunity against *C. albicans* and is cross-protective against MDR *C. auris* and GNB (AB, KP, PA). Further development of this novel CKV targeting multiple priority MDR pathogens is warranted.