

Immunodetection and immunotherapy of *Candida albicans* infection using anti-alpha-enolase antibody

利用抗- α 異烯醇酶抗體來免疫偵測與免疫治療
白色念珠菌感染

呂思潔

微生物及免疫學科

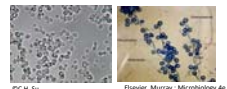


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Candida albicans

- Candida* yeast is an opportunistic human pathogen
- Candida* infections have increased dramatically due to immunosuppressive therapy, long-term catheterization, use of broad-spectrum antibiotics and longer survival of immunocompromised individuals.
- Candida* infections occur through bloodstream dissemination and range from superficial to systemic diseases
- C. albicans* also invades the central nervous system, resulting in devastating meningitis

(CDC – Antibiotic Resistance Threats in the United States, 2015)
(J Clin Microbiol Rev. (2007))



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Invasive candidiasis

- Invasive candidiasis has been one of the important issues in clinical settings and has been a leading cause of health care-associated infections, resulting in increased length and cost of hospitalizations as well as morbidities/mortalities of patients.
- In the US, Invasive candidiasis is one of the most common bloodstream infections, and is estimated that approximately 46,000 cases of healthcare-associated invasive candidiasis occur each year.
- In Taiwan, candidiasis is the second most common cause of health care-associated bloodstream infections ranking only after bacteremia.

(CDC – Antibiotic Resistance Threats in the United States. (2013))
(J MICROBIOL IMMUNOL. (2015) p. 306-315)



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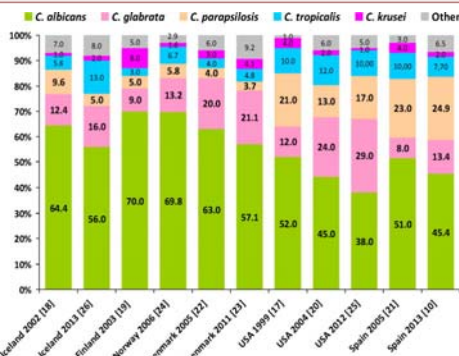
Increased infection of *Candida* spp.

- Increased antibiotic resistance strains of bacteria
- Increased patients with weak immune system
 - Organ transplantation
 - Stem cell transplantation
 - Cancer patients with chemotherapy
 - AIDS
- Increased patients in critical care medicine



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Global Trends in the Distribution of *Candida* spp.



Clin Microbiol Infect. 2014 Jun;20 Suppl 6:5-10



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ANTIBIOTIC RESISTANCE THREATS in the United States, 2013

HAZARD LEVEL URGENT

These are high-consequence antibiotic-resistant threats because of significant risks identified across several criteria. These threats may not be currently widespread but have the potential to become so and require urgent public health attention to identify infections and to limit transmission.

Clostridium difficile (*C. difficile*), Carbapenem-resistant Enterobacteriaceae (CRE), Drug-resistant *Neisseria gonorrhoeae* (cephalosporin resistance)

HAZARD LEVEL SERIOUS

These are significant antibiotic-resistant threats. For varying reasons (e.g., low or declining domestic incidence or reasonable availability of therapeutic agents), they are not considered urgent, but these threats will worsen and may become urgent without ongoing public health monitoring and prevention activities.

Multidrug-resistant *Acinetobacter*, Drug-resistant *Compylobacter*, Fluconazole-resistant *Candida* (a fungus), Extended spectrum β -lactamase producing Enterobacteriaceae (ESBLs), Vancomycin-resistant *Enterococcus* (VRE), Multidrug-resistant *Pseudomonas aeruginosa*, Drug-resistant Non-typhoidal *Salmonella*, Drug-resistant *Salmonella* Typhi, Drug-resistant *Shigella*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Drug-resistant *Streptococcus pneumoniae*, Drug-resistant tuberculosis (MDR and XDR)

HAZARD LEVEL CONCERNING

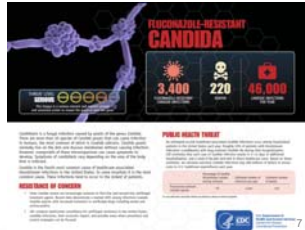
These are bacteria for which the threat of antibiotic resistance is low, and/or there are multiple therapeutic options for resistant infections. These bacterial pathogens cause severe illness. Threats in this category require monitoring and in some cases rapid incident or outbreak response.

Vancomycin-resistant *Staphylococcus aureus* (VRSA), Erythromycin-resistant *Streptococcus* Group A, Clindamycin-resistant *Streptococcus* Group B

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Drug Resistance Problems

- **Amphotericin (AmB)**
 - gold standard of antifungal treatment
 - a narrow therapeutic window and significant adverse effects, especially nephrotoxicity
- **Fluconazole**
 - one of most prescribed antifungal drugs in ICU
 - clinically significant drug interactions
 - elevation of hepatic transaminases
 - gastrointestinal tract adverse effect
- **Echinocandins**
 - newest class of antifungal agents
 - primary treatment option for invasive candidiasis
 - high cost in treatment



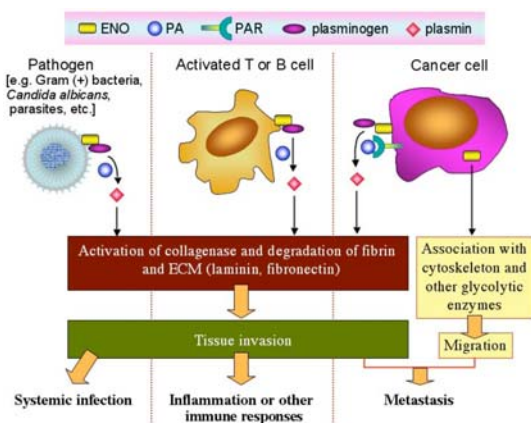
α 異烯醇酶 [α -Enolase (Eno1)]

- Eno1 is a key glycolytic enzyme in the cytoplasm of prokaryotic and eukaryotic cells and is considered a multifunctional protein.
- Eno1 is expressed on the surface of several cell types, where it acts as a plasminogen receptor, and driving tumor invasion through plasminogen activation and ECM degradation.
- Expression of Eno1 has been related to several pathologies, such as cancer, autoimmune disorders, ischaemia and bacterial infection.



[Angels Diaz-Ramos et al., 2012]

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[Liu and Shih. J. Cancer Mol., 2007]

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Candida albicans Eno1 (CaEno1)

- *Candida albicans* eno1 null mutants exhibit altered drug susceptibility, germ-tube and hyphal formation, and virulence.
- The expression of Eno1 in the fungal pathogen *Candida albicans* is critical for cell growth.
- Mutations on Eno1 in *Candida albicans* inhibit cell growth in the presence of glucose.
- *In vivo*, increased levels of fungal-specific enolase have also been found in patients with invasive candidiasis.

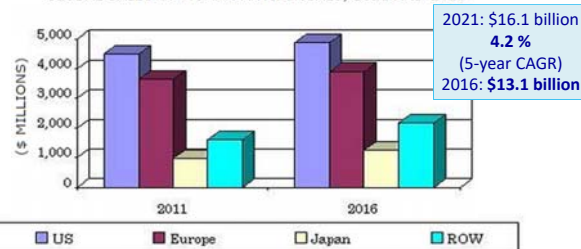


[J Microbiol. 2013 Jun;51(3):345-51]
[Journal of Biomedical Science (2006) 13:313-321]
[Angels Diaz-Ramos et al., 2012]

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Global Market For Antifungal Therapeutics

GLOBAL SALES OF HUMAN ANTIFUNGALS, 2011 AND 2016



\$4.5 billion/2011 projected \$4.9 billion/2016 CAGR: 1.7%
\$3.6 billion in 2011 projected to \$3.9 billion/2016 CAGR: 1.5%
\$963 million/2011 expected 1.2 billion/2016 CAGR: 5.3%
\$1.6 billion/2011 projected 2.2 billion/2016 highest CAGR: 6.3%



Source: BCC Research

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Development of Monoclonal Antibody (mAb)

- Even with treatment using available antifungal agents, morbidities/mortalities is still very high!
- Emerging bloodstream infection have antibiotics/antifungal agent resistance problem.
- Hospitalizations for invasive candidiasis in recovery during and cost is high.
- To assess the potential of monoclonal antibody target to Eno1 of *C. albicans* as a potential effective therapeutic treatments or diagnostic agents against *C. albicans* infection



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Aims

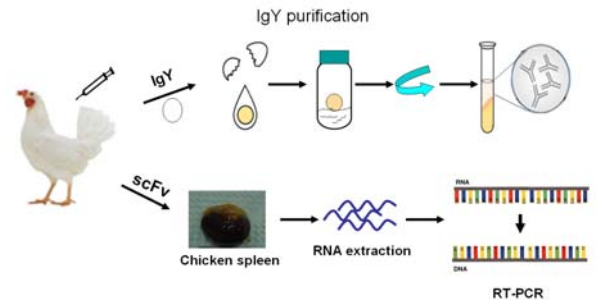
1. Production of polyclonal and monoclonal Ab against CaEno1 by phage display
2. Expression, purification and characterization of CaS1 scFv
3. Epitope mapping of CaEno1 with CaS1 scFv
4. Investigation of the interaction between CaEno1 and plasminogen
5. Animal model of *C. albicans* infection



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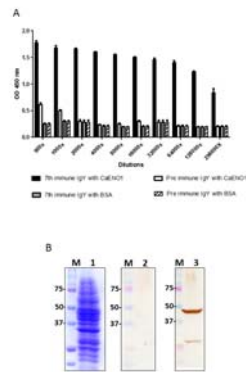
Immunization and IgY purification

雞隻的免疫注射與IgY純化



Core Laboratory of Antibody Generation and Research, TMU

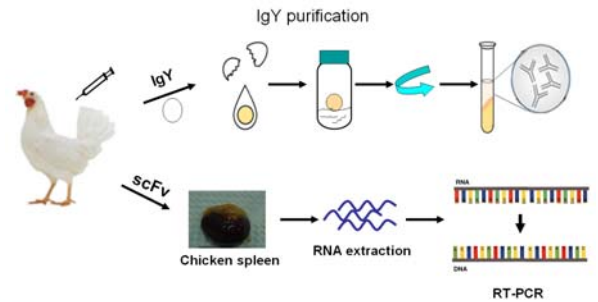
Humoral antibody response in chickens



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Immunization and IgY purification

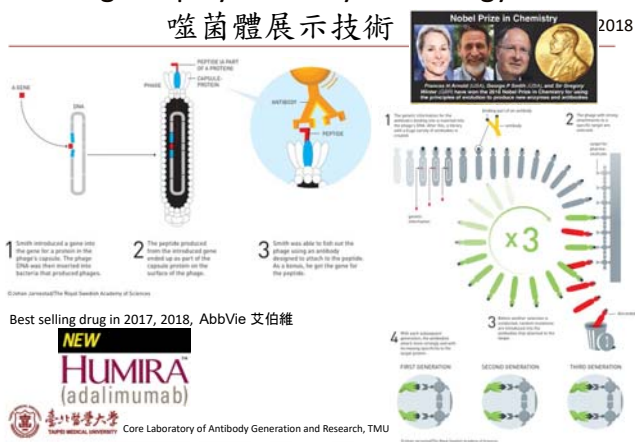
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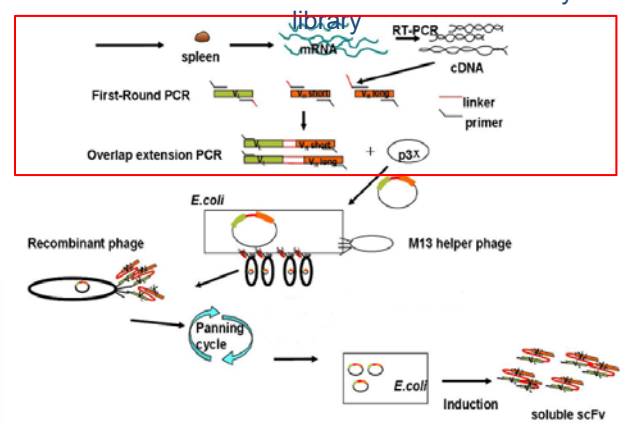
Phage display antibody technology

噬菌體展示技術



Core Laboratory of Antibody Generation and Research, TMU

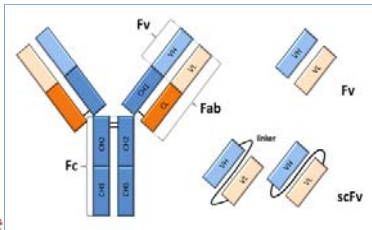
Construction and selection of scFv antibody



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Single Chain Variable Fragment (scFv)

- A small engineered antibody, in which the variable heavy chain (V_H) and light chain (V_L) of the antibody molecule are connected by a short, flexible polypeptide linker.



Monoclonal scFv Production

Table 1. The anti-CaEno1 library size and eluted phage titers after each round of panning.

Library	Linker length*	Library size	Eluted phage titers after each round of panning			
			1 st	2 nd	3 rd	4 th
CaEno1-5	7 aa	2.4×10^6	9.6×10^4	9.0×10^5	7.2×10^5	3.0×10^5
CaEno1-4	18 aa	1.36×10^7	2.75×10^5	1.2×10^6	1.32×10^6	1.2×10^5

*Linker length of 7 aa and 18 aa are GQSSRS5 and GQSSRS5GGGGSSGGGG5, respectively.

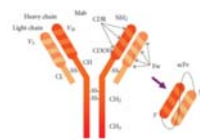
- We constructed two libraries: short linker (2.4×10^6) and long linker (1.36×10^7)
- One scFv antibody (CaS1) with specific binding ability against CaEno1 was isolated after biopanning



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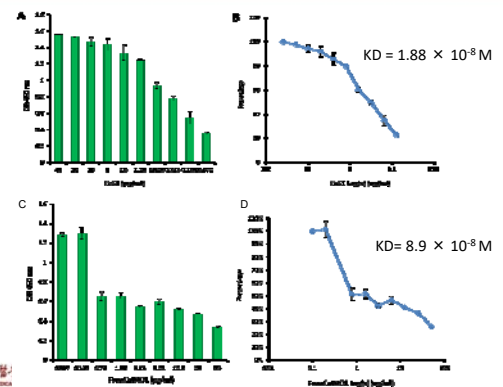
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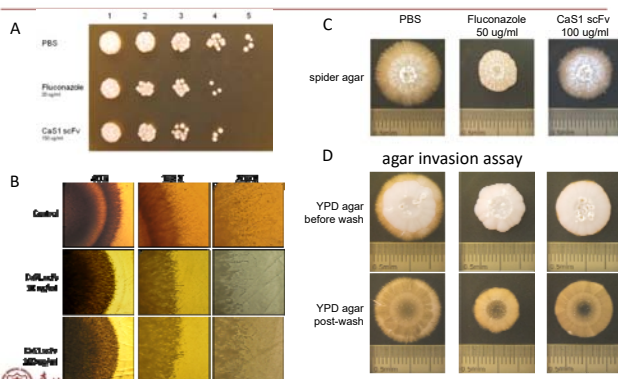
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KD determination of CaS1 scFv against CaEno1 by ELISA and competitive ELISA



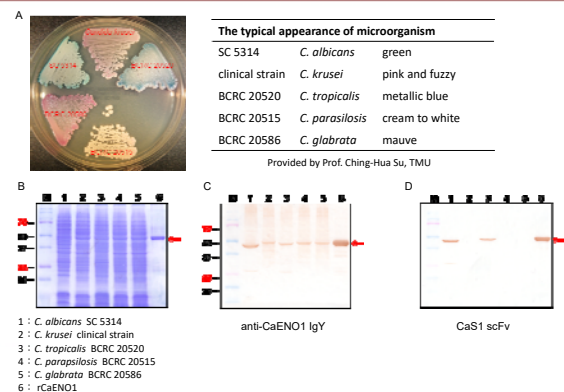
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CaS1 scFv reduced colony and hyphae formation



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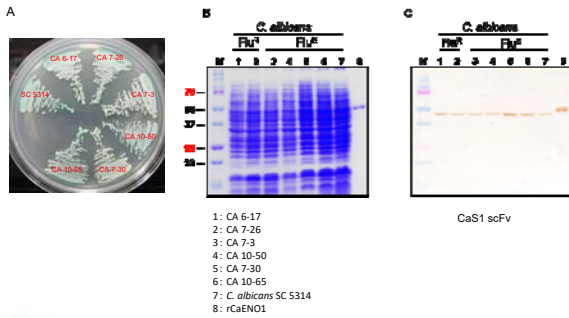
Binding of anti-CaEno1 IgY and CaS1 scFv against *Candida* species by Western blot



- C. albicans* SC 5314
- C. krusei* clinical strain
- C. tropicalis* BCRC 20520
- C. parapsilosis* BCRC 20515
- C. glabrata* BCRC 20586
- rCaEno1

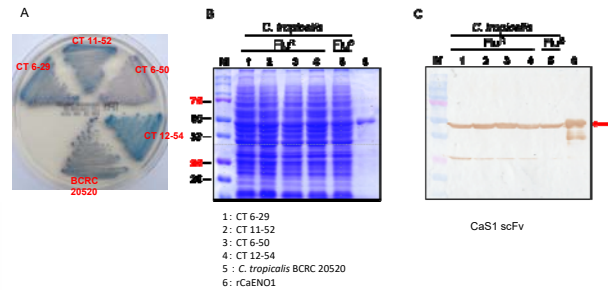
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Binding of CaS1 scFv against *C. albicans* clinical strains by Western blot



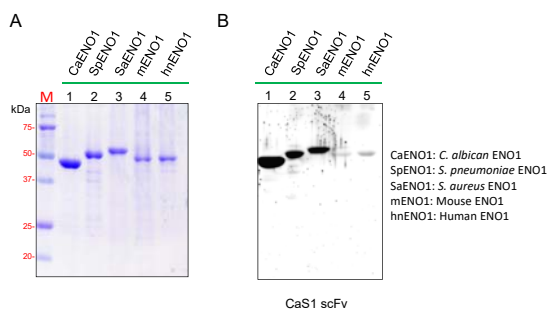
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Binding of CaS1 scFv against *C. tropicalis* clinical strains by Western blot



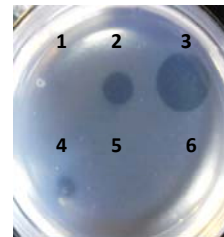
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Comparison of binding reactivity by CaS1 scFv with different species Eno1



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CaS1 scFv inhibited CaEno1 binding to plasminogen by matrix-gel degradation assay



1. *C. albicans* only
2. *C. albicans* + 1 μ g plasminogen
3. *C. albicans* + 10 μ g plasminogen
4. *C. albicans* + 10 μ g CaS1 scFv + 10 μ g plasminogen
5. *C. albicans* + 100 μ g CaS1 scFv + 10 μ g plasminogen
6. *C. albicans* + CaS1 scFv as a NC

Fibrin matrix-gel degradation analysis
Gel contains :

low-melting-temperature agarose (1.25 %)
thrombin (0.05 U/ml)
fibrinogen (2 mg/ml)
emulsifying (50 μ g/ml)

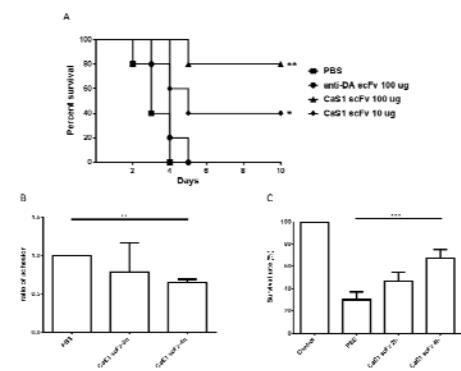
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Aims

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CaS1 prolong the survival rate of mice and zebrafish infected with *C. albicans*



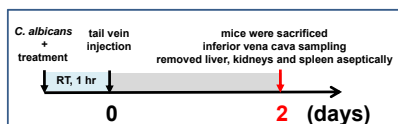
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Mouse model of *C. albicans* infection



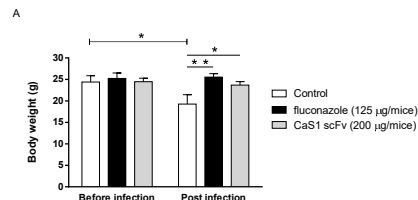
C. albicans, SC 5314 (1×10^6 cfu/mice)

- A: PBS
- B: Fluconazole (125 µg/mice)
- C: CaS1 scFv (200 µg/mice)
- D: hz CaS1 IgG V1 (200 µg/mice)



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Body weight measurement of *C. albicans* infected mice

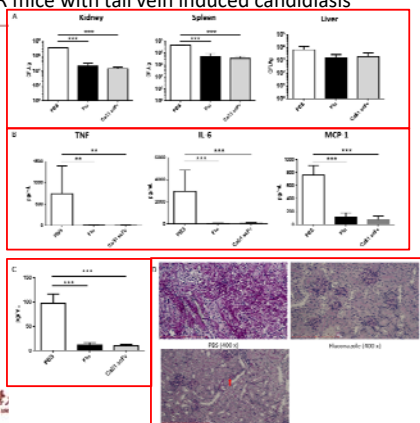


group	<i>C. albicans</i> SC 5314 (cfu)	treatment	dose (µg/mice)	weight (g) before infection	weight (g) post infection
A	1×10^6	PBS	-	24.40 ± 3.27	19.27 ± 3.79
B	1×10^6	fluconazole	125	24.40 ± 5.29	$25.54 \pm 1.82^{**}$
C	1×10^6	CaS1 scFv	200	24.48 ± 1.79	$23.70 \pm 1.79^*$



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CaS1 reduced on fungal burden and cytokine levels on ICR mice with tail vein induced candidiasis



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Conclusions



- Anti-α-enolase monoclonal antibody (CaS1) was predominantly identified using phage display antibody technology.
- CaS1 scFv was constructed, expressed and shown binding activity to CaEno1 (KD: 10^{-8} M).
- CaS1 scFv bind to Eno1 protein of standard strains and clinical strains of *C. albicans* and *C. tropicalis*, but not to *C. krusei*, *C. parapsilosis* and *C. glabrata*.
- CaS1 scFv bind to Eno1 protein of *C. albicans*, *S. pneumoniae*, *S. aureus*, have weak cross reactivity to mouse and human.
- CaS1 showed potent anti-*C. albicans* properties *in vitro*
- CaS1 prolonged survival rate and decreased fungal burden, inflammatory cytokines and β-D-glucan level in animal models.



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Conclusions



- CaS1 could be a starting point for further development of fungal-specific therapies.
- CaS1 provides an alternative immunotherapy for the treatment of candidiasis caused by *C. albicans* infection.



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Acknowledgments

TMU

Yi-Yuan Yang Ph.D.
Yu-Ching Lee Ph.D.
Ching-Hua Su Ph.D.
Chi-Hsin Lee Ph.D.
Po-Yen Liao
Chen-Wei Chiang
Chieh-Ming Yang

Charles Chen DVM

WFH

Tsong-Yih Ou MD
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Ko-Jiunn Liu Ph.D.
Hsiu-Jung Lo Ph.D.

NRICM

Keng-Chang Tsai Ph.D.

NAVI BIO-THERAPEUTICS INC.

Bor Yu Tsai Ph.D.



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