Treatment of Aspergillosis, Candidiasis, and Cryptococcosis with DectiSomes

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Abstract

DectiSomes are lipid nanoparticles (e.g., liposomes) carrying an anti-infective drug and coated with pathogen receptor protein that targets them to pathogenic cells.[1] We have demonstrated the pan-antifungal efficacy of DectiSomes by using the carbohydrate recognition domains (CRDs) of three C-type lectin pathogen receptors Dectin-1 (CLEC7A), Dectin-2 (CLEC4N), and the DCS12 isoform of DC-SIGN (CD209) to target Amphotericin B loaded liposomes to Aspergillus fumigatus, Candida albicans, and Cryptococcus neoformans. These three pathogens represent 1.2 billion years of fungal pathogen evolution and divergence. The three classes of DectiSomes, DEC1-AmB-LLs[2], DEC2-AmB-LLs[3], and DCS12-AmB-LLs[4], bound all three species order(s) of magnitude better than control liposomes, including untargeted AmB-LLs (analogous to Ambisome®) and bovine serum albumin coated BSA-AmB-LLs. The various DectiSomes killed these fungal pathogens in vitro order(s) of magnitude more efficiently than control liposomes and lowered the effective dose ED90 10- to 20-fold [2,3,4]. Oral aspiration and intravenous injection, respectively, of DectiSomes reduced the fungal burden of A. fumigatus in the lungs[5] and C. albicans in the kidneys[6] 10-fold compared to the untargeted control liposomes. DectiSomes improved the survival of mice with pulmonary aspergillosis[5] and invasive candidiasis[6]. Using the combination of novel and synthetic techniques we have developed, the production of C-type lectin-targeted DectiSomes is 1,000-times less expensive than monoclonal targeted immunoliposomes[1]. Low cost and dramatically improved efficacy should encourage pharmaceutical companies to commit time and resources to the clinical development of DectiSomes as pan-antifungal therapeutics.

Publications

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2. Ambati, S., Ferarro, A.R., Kang, S.E., Lin, J., Lin, X., Momany, M., et al. Dectin-1-Targeted Antifungal Liposomes Exhibit Enhanced Efficacy. mSphere 4, 1-15 (2019). 10.1128/mSphere.00025-19. https://www.ncbi.nlm.nih.gov/pubmed/30760610.
3. Ambati, S., Ellis, E.C., Lin, J., Lin, X., Lewis, Z.A. & Meagher, R.B. Dectin-2-Targeted Antifungal Liposomes Exhibit Enhanced Efficacy. mSphere 4, 1-16 (2019). 10.1128/mSphere.00715-19. https://www.ncbi.nlm.nih.gov/pubmed/31666315.
4. Ambati, S., Pham, T., Lewis, Z.A., Lin, X. & Meagher, R.B. DC-SIGN Targets Amphotericin B-Loaded Liposomes to Diverse Pathogenic Fungi. Fungal Biol and Biotech 8, 1-13 (2021). https://doi.org/10.1186/s40694-021-00126-3. https://rdcu.be/cDPZS.
5. Ambati, S., Ellis, E.C., Pham, T., Lewis, Z.A., Lin, X. & Meagher, R.B. Antifungal Liposomes Directed by Dectin-2 Offer a Promising Therapeutic Option for Pulmonary Aspergillosis. mBio 12, 1-8 (2021). 10.1128/mBio.00030-21. https://www.ncbi.nlm.nih.gov/pubmed/33622715.
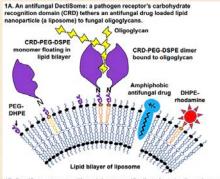6. Ambati, S., Pham, T., Lewis, Z.A., Lin, X. & Meagher, R.B. DectiSomes- Glycan Targeting of Liposomal Amphotericin B Improves the Treatment of Disseminated Candidiasis. Antimicrob Agents Chemother (2021). https://journals.asm.org/doi/10.1128/AAC.01467-21.

Fig. 1. DectiSomes are liposomes designed to target antifungal drugs specifically to fungal cells. A. Design of a DectiSome. One iteration of a DectiSome is an Amphotericin B-loaded liposome coated with the glycan recognition domain of Dectin-1. The Dectin-1 monomers float freely and form even more active multimers that bind their cognate ligands. B. Specificity of a DectiSome. DectiSomes concentrate liposomal drugs on fungal cells and their exopolysaccharide matrices (right side). Current antifungal drugs are un-targeted (left side) and distribute drugs almost equally among fungal cells and host cells. Infection centers with yeast (Y) or filamentous (F) cell morphologies are shown.

C-Type Lectin Pathogen Receptor (gene)	Cognate ligands	Targeted pathogens that were tested
Dectin-1 (CLEC7A)	beta-glucan oligomers	A. fumigatus, C. albicans, C. neoformans
Dectin-2 (CLEC4N),	alpha-mannan oligomers, manno-proteins, and mannose-capped lipoarabinomannan ManLAM	A. fumigatus, C. albicans, C. neoformans
DC-SIGN (CD209) Isoforms DCS12 and DCS78 have different neck repeats.	mannose-rich and fucosylated glycans (e.g., the Lewis[X] trisaccharide), ManLAM, and lipomannans often found in protein conjugates	A. fumigatus, C. albicans, C. neoformans

Table 1. C-Type Lectin Pathogen Receptors we've shown successfully target DectiSomes to diverse fungal pathogens.
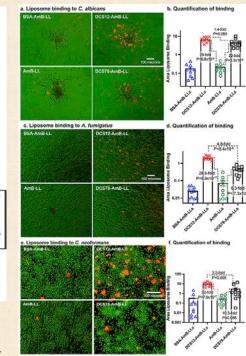

Fig. 2. DC-SIGN targeted liposomes bind efficiently to three fungal pathogens: examples of DectiSome specificity. Liposomes loaded with AmB and targeted by two isoforms of human DC-SIGN, DCS12-AmB-LLs and DCS78-AmB-LLs, bound more efficiently and significantly to the exopolysaccharide matrices of three fungal pathogens relative to untargeted AmB-LLs or BSA coated BSA-AmB-LLs. a, c, e. Representative photographic images of red fluorescent liposomes binding to bright field images of fungal cells. a. C. albicans (10X magnification). c. A. fumigatus (10X magnification). e. C. neoformans (20X magnification). b, d, f. The relative area of red fluorescent liposome binding (log$_{10}$) was quantified as shown in scatter bar plots on the right. f. The scale of the plot for C. neoformans had to be expanded from three to five logs to accommodate more widely distributed data. Standard errors and P values are indicted. N=10 images for each bar. In general, the DCS12 isoform performed better than the DCS78 isoform.
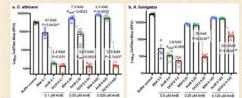

Fig. 3. A metabolic activity assay shows DCS12-AmB-LLs were order(s) of magnitude more effective at inhibiting and/or killing C. albicans and A. fumigatus in vitro than untargeted liposomal AmB (AmB-LLs) and significantly reduced the effective dose of AmB.

Fig. 4. Intravenous & oropharyngeal delivery of DCS12-AmB-LLs dramatically reduced the murine organ fungal burden for two fungal pathogens & reduced the effective dose of AmB.
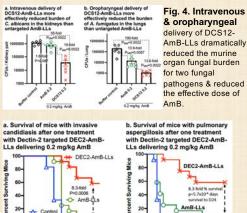

Fig. 5. Intravenous & oropharyngeal administration of Dectin-2 targeted DectiSomes, DEC2-AmB-LLs delivering a very low dose of AmB (0.2 mg/kg) significantly improved the survival of mice with (a) invasive candidiasis and (b) pulmonary aspergillosis.

Summary and Conclusions.

1. DectiSomes are anti-infective drug loaded liposomes targeted by pathogen receptor CRDs.
2. We've shown that the CRDs from three mammalian pathogen receptors Dectin-1, Dectin-2, and DC-SIGN all are effective liposome targeting polypeptides.
3. These DectiSomes recognize pathogens representing much of the diversity in the fungal kingdom.
4. DectiSomes delivering relatively low doses of AmB inhibit and/or kill these fungi in vitro.
5. DectiSomes delivering relatively low doses of AmB dramatically improve mouse survival.
6. DectiSomes are estimated to be 1,000-fold less expensive to produce that immunoliposomes.