Oral presentation

Exploring the antifungal potential of Bdf1 bromodomain inhibitors

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Background. BET (Bromodomain and Extra-Terminal) proteins are chromatin-associated proteins that recognize acetylated histones via their two bromodomains (BDs). Small molecules that target the BDs of human BET proteins are intensely pursued as potential therapeutics against cancer and other diseases. We previously identified the fungal BET protein Bdf1 as a potential antifungal target against invasive fungal infections [1]. However, the need to selectively inhibit both Bdf1 BDs over human orthologs and the lack of molecular tools to assess on-target antifungal efficacy have hampered efforts to develop Bdf1 BD inhibitors as antifungal therapeutics.

Methods. We explored Bdf1 BD inhibition in the human fungal pathogen *Candida glabrata*. We generated inactivating mutations of the protein's two BDs, used chemical screening to identify Bdf1 BD inhibitors, profiled their potency and selectivity in inhibition assays *in vitro*, determined crystal structures of inhibitor-bound BDs, tested inhibitors for antifungal activity against diverse *Candida* species *in vitro* and in an invertebrate (*Galleria mellonella*) model of infection, and verified on-target activity in novel yeast-based assays.

Results. We found that the mutational inactivation of both Bdf1 BDs is lethal in *C. glabrata*. Chemical screening followed by hit optimization identified a phenyltriazine compound that inhibits both Bdf1 BDs with selectivity over human BET BDs. We established on-target antifungal activity by devising two yeast-based inhibition assays: a growth assay using humanized *Candida* strains in which the Bdf1 BDs are replaced by their human counterparts, and a NanoBiT assay that evaluates the BD-mediated association of Bdf1 with chromatin (Fig. 1). These assays additionally enabled the discovery that BET inhibitor I-BET726 targets both Bdf1 BDs, inhibits the growth of diverse *Candida* species, including antifungal-resistant clinical isolates, and displays efficacy in the *Galleria* model of infection. Crystal structures of inhibitor-bound Bdf1 BDs revealed enlarged binding pockets that suggest how to enhance inhibitor selectivity and potency.

Conclusions. Our findings provide compelling support for the development of Bdf1 BD inhibitors as an innovative class of antifungal therapeutics, while highlighting the pivotal role of yeast-based assay development towards realizing this goal.

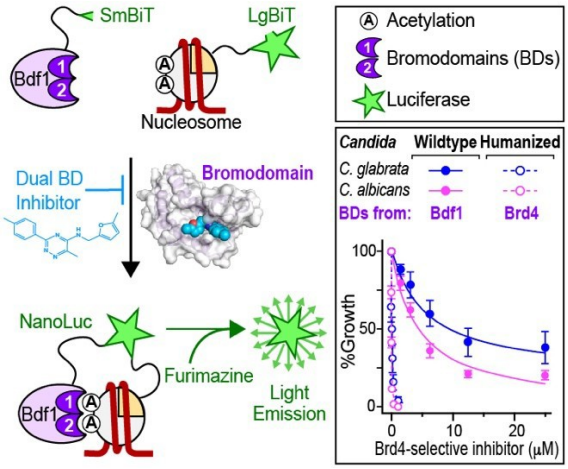

Figure 1. Fungal protein Bdf1 is a potential antifungal target in pathogenic *Candida* species requiring the inhibition of both its bromodomains. Humanized *Candida* strains and NanoBiT assays developed to evaluate on-target antifungal efficacy expedited the discovery of Bdf1 inhibitors with antifungal potential.

[1] Mietton, F., Ferri, E., Champleboux, M., Zala, N., Maubon, D., Zhou, Y., Harbut, M., Spittler, D., Garnaud, C., Chauvel, M., d'Enfert, C., Kashemirov, B.A., Hull, M., Cornet, M., McKenna, C.E., Govin, J. & Petosa, C. (2017) *Nature Comm.*, **8**,15482.