

EMERGING ECHINOCANDIN RESISTANCE – A REVIEW

Abstract :Worldwide there is a steady increase in the number of fungal infections each year. Simultaneously, there is a high rise of antifungal resistance among the *Candida* isolates. This has now become a major concern in hospitalized patients resulting in poor treatment outcome. Echinocandins, a new group of antifungals has given promising results inspite of its increasing rate of resistance exhibited by some *Candida* species.

INTRODUCTION: There is an increasing incidence of fungal infections worldwide(1).Antifungal therapy is the major treatment option for patients suffering from fungal infections. But treatment options are limited owing to the fewer antifungal drugs and resistance to the existing antifungal drugs. The increased use of antifungals for candidiasis has led in the development of resistant *Candida* isolates(2).Azoles antifungals and Amphotericin B are a cornerstone for therapy till now. Toxicities associated with amphotericin and increasing azole resistance have urged the need for an alternative replacement in the management of fungal infections(3).Echinocandins, developed over a decade is a milestone in antifungal therapy. Currently, echinocandins are the first line choice for systemic *Candida* infections and a majority of patients with candida blood stream infections are on echinocandin therapy(4,5).

This article will highlight list the available echinocandin drugs available, their mode of action ,the acquired resistance mechanisms and its clinical implications.

ECHINOCANDINS – NEW CLASS OF ANTIFUNGALS:

Three echinocandin antifungal drugs caspofungin, micafungin, and anidulafungin are available over a decade.. The echinocandins have a distinctive mechanism of action, suppressing the action of (1,3)-D-glucan synthase (6).Echinocandins have been used in invasive candidiasis. In addition, caspofungin is used in febrile neutropenia and invasive aspergillosis, and is safe for use in pediatric patients (7). Micafungin is the only echinocandin used in bone marrow transplantation (8).

SPECTRUM OF ACTIVITY :

Echinocandins exhibit good fungicidal activities against candida spp mainly *Candida albicans*, *Candida parapsilosis* and *Candida guilliermondii*. Good activity is also shown against amphotericin B-resistant and fluconazole-resistant *Candida glabrata*(9,10). For Aspergillus species, echinocandins have fungistatic activity in contrast to amphotericin B and triazoles, which exhibit fungicidal activity. Echinocandin resistance, though not common, has been reported in *C. glabrata* and *C. parapsilosis*.(11,12)

CRITERIA AND CUTOFF FOR RESISTANCE :

Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have established standardized microbroth dilution susceptibility tests for *Candida* and echinocandins which show uniformly potent activity against most *Candida* species”(13-15). “Both CLSI and EUCAST have confirmed species and drug- specific clinical breakpoints (CBP) for echinocandin drugs and epidemiological cutoff values have been defined for anidulafungin and micafungin against common *Candida* species”(16-19). “EUCAST has not established caspofungin breakpoint and does not recommend caspofungin E test for MIC detection or sensititre yeast only system for clinical assessment, owing to high interlaboratory testing variability and the CLSI has raised caution when using caspofungin testing, especially with *C. glabrata*”(20,21). Many of the candida isolates showing resistant to caspofungin were susceptible to anidulafungin and micafungin(21,22).owning to this EUCAST has gauged anidulafungin and micafungin as markers for caspofungin susceptibility(23,24).

ACQUIRED RESISTANCE MECHANISMS :

FKS Mechanism of Resistance

Aminoacid alteration in the Fks subunits of glucan synthase causes echinocandin resistance(25). “Mutations are highly confined to Phe641–Pro649 and Arg1361 (*C. albicans* equivalent) and/or equivalent regions of *FKS2* in *C. glabrata* (26,27)”. “Amino acid substitutions in Fks subunits induce elevated MIC values (10–100 fold) and reduce the sensitivity of glucan synthase (IC₅₀) to drug by as much as 3000-fold”(28). Amino acid alterations are more frequent in Fks 2 than Fks 1 (26,29,30). These mutations in Fks region **alter** the pharmacodynamic profile of the drug (31,32).In *C. albicans*, changes at position Ser645 is the most common resistant pattern noticed(29). In *C. glabrata*, mutations are common in *FKS1* and *FKS2*. “Changes located at Ser663 (equivalent

to *C. albicans* Ser645) in Fks2 is the commonest”(28,33).other interchanges at Ser629 in Fks1 and Phe659 in Fks2 also result in treatment failure (34). “Other hot-spot mutations may confer phenotypic resistance, but escalating drug doses are more effective against resistant strains harboring such mutations”(35). Presence of mutations in Fks 1 or Fks 2 along with the gene expressions determines the resistance.(30,36,37) *C. tropicalis*, *C. krusei* and *C. kefyr* confer resistance to echinocandins by Fks mechanism.(38,39)

Hot spot diversity

Mutation at Phe641 and Ser645 in *C. albicans* is most commonly noticed compared to the C-terminal end of hot spot 1, which is less commonly noticed (30,40,41). Diversity at Pro649 in the *C. parapsilosis* and at Met633 and Ala634 in *C. guilliermondii*, causes increased MIC values(42). Infecting strains with intrinsically reduced susceptibility carries an uncertain clinical significance (43,44).The sensitivity of glucan synthase for echinocandins is lower in candida parapsilosis than candida albicans which causes high MIC value. “But the enzyme, while less sensitive, is still inhibited at typical therapeutic drug concentrations, which accounts for clinical response”(45). “Mutations at the third region W695 (outside clinical hot spots 1 and 2) of *Saccharomyces cerevisiae* Fks1 is found but does not cause treatment failure”(46).

Biofilms

Biofilms are “a thin but robust layer of mucilage adhering irreversibly to a solid surface, inert material, or living tissue producing extracellular polymers that provide a structural matrix and contain a community of bacteria and other microorganisms (47,48)”. *Candida* species have inherent tendency to form biofilms. The beta 1,3 D glucan a component of biofilm matrix seizes the drug and allows less concentration at cellular level(49).Alteration of the transcriptional regulators like R1m and Sml1 and changes in the Fks alter the glucan synthesis which in turn leads to the formation of biofilms conferring resistance (50).

Adaptive Cellular Factors

All fungi possess various factors to overcome cellular stress.On encountering a cellular stress there is increased MIC level of the drug which does not always correlate with treatment response (25).This is the preliminary state for Fks mechanism of resistance and in turn lead to full blown resistance. Fungal stress adaptive pathways lead to increased production of chitin as a compensatory mechanism leading to resistance (51).This acts at the level of cell wall. Changes in the cell membrane level can also alter the activity of echinocandin. Alteration in the

sphingolipid synthesis and composition alters the efficacy of caspofungin and micafungin (52).

Hsp90

Heat shock proteins are “a family of proteins that are produced by cells in response to exposure to stressful conditions (53)”. Hsp90 changes are not only associated with azole resistance but also with echinocandin resistance. Any reduction in the function of Hsp90 causes resistance in *C. albicans*, *C. glabrata*, and *A. fumigatus* (54,55). Hsp90 confers resistance to echinocandins by calcineurin and Mkc1. Inhibition of Hsp90 activity increases the efficacy and activity of echinocandins. This clearly suggests Hsp90 acts as a target to increase the potency of echinocandins (56).

| ECHINOCANDIN DRUG RESISTANCE | |
|------------------------------|-------------------------------------------------------------------------------------------------------|
| Mechanism of Resistance | Fungal species |
| FKS | <i>C. albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i> , <i>C. krusei</i> and <i>C. kefyr</i> |
| Heat Shock Protein 90 | <i>C. albicans</i> , <i>C. glabrata</i> , and <i>A. fumigatus</i> |
| Hot Spot Diversity | <i>C. parapsilosis</i> , <i>C. guilliermondii</i> |
| Biofilm | All <i>Candida</i> species |

CLINICAL IMPLICATIONS :

A huge population of patients suffering from systemic fungal infections do not respond to antifungal therapy due to acquired drug resistance (57). In vivo success therapy with antifungal drugs was found to be less than 75% compared to the in vitro susceptibility testing results indicating in vitro results alone cannot predict treatment outcome (58,59).

Though in recent times there is emergence of echinocandin resistant *Candida* species, resistance noticed in *Candida albicans* and some of the other *Candida* spp is still low varying from 2-3%. Contrary to this the level of resistance in *Candida glabrata* is very high ranging from 8-13%. Increased use of echinocandin for *Candida glabrata* infections has alarmingly increased resistance from 3% to >13 (60,61). In many cases these echinocandin resistant isolates are also azole resistant leading to multidrug resistant *Candida* spp imposing a treatment challenge (62).

CONCLUSION :

Echinocandins in contrast to the other class of antifungals possess several

unique merits. when choosing such a drug for patient therapy the treating physician should take in to account the metabolism and drug interaction, the dose, duration to be prescribed and their recommended indications

REFERENCES:

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC
1. Sci Transl Med. 2012 Dec 19; 4(165):165rv13
2. Antonovics J, Abbate JL, Baker CH, Daley D, Hood ME, Jenkins CE, Johnson LJ, Murray JJ, Panjeti V, Rudolf VH, et al. 2007. Evolution by any other name: Antibiotic resistance and avoidance of the E-word. PLoS Biol 5: e30.
3. Grover ND. Echinocandins: A ray of hope in antifungal drug therapy. *Indian J Pharmacol*. 2010;42(1):9-11. doi:10.4103/0253-7613.62396
4. Pappas PG, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2009; 48:503–535.
5. Cleveland AA, et al. Changes in incidence and antifungal drug resistance in candidemia: results from population-based laboratory surveillance in Atlanta and Baltimore, 2008–2011. *Clin Infect Dis*. 2012; 55:1352–1361.
6. Onishi J, et al. Discovery of novel antifungal (1,3)-beta-D-glucan synthase inhibitors. *Antimicrobial Agents and Chemotherapy*. 2000; 44:368–377.
7. Caspofungin prescribing information. Whitehouse Station NJ: Merck and Co; Available from: http://www.cancidas.com/caspofungin_acetate/cancidas/hcp/product_information/pi/index.jsp
8. Micafungin prescribing information. Astellas Pharma US. 2005. Apr, Available from: <http://www.mycamine.com/pi.php>
9. Barchiesi F, et al. Comparison of the fungicidal activities of caspofungin and amphotericin B against *Candida glabrata*. *Antimicrobial Agents and Chemotherapy*. 2005; 49:4989–4992.
10. Ernst EJ, et al. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. *Diagn Microbiol Infect Dis*. 1999; 33:75–80.
11. Bowman JC, et al. Efficacy of caspofungin against *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus nidulans*. *Antimicrobial Agents and Chemotherapy*. 2006; 50:4202–4205.
12. Bowman JC, et al. The antifungal echinocandin caspofungin

- acetate kills growing cells of *Aspergillus fumigatus* in vitro. *Antimicrobial Agents and Chemotherapy*. 2002; 46:3001–3012.
13. Clinical and Laboratory Standards Institute (CLSI). CLSI document M27-A3. Clinical and laboratory Standards Institute; Pennsylvania, USA: 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard - Third edition. [L1] [SEP]
 14. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope W. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). *Clin Microbiol Infect*. 2012 Jul; 18(7):E246–E247.
 15. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. An Update from EUCAST focussing on Echinocandins against *Candida* spp. and Triazoles Against *Aspergillus* spp. *Drug Resist Updat*. 2014 In press. Comprehensive overview of the science and methodologies behind EUCAST clinical breakpoint development. Provide the reader with an understanding of the pitfalls related to susceptibility testing, the recent advances and how this may be translated to the routine laboratory using commercial tests.
 16. Marcos-Zambrano LJ, Escribano P, Sanchez C, et al. Antifungal Resistance to Fluconazole and Echinocandins Is Not Emerging in Yeast Isolates Causing Fungemia in a Spanish Tertiary Care Center. *Antimicrobial Agents and Chemotherapy*. 2014 Aug
 17. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. EUCAST Technical Note on Candida and Micafungin, Anidulafungin and Fluconazole. *Clin Microbiol Infect*. 2013 In Press. provide the new micafungin and the revised anidulafungin and fluconazole breakpoints from EUCAST.
 18. Clinical and Laboratory Standards Institute (CLSI). CLSI document M27-S4. Clinical and laboratory Standards Institute; Pennsylvania, USA: 2012. Reference method for broth dilution antifungal susceptibility testing of yeasts; Fourth informational Supplement.
 19. Arendrup MC, Dzajic E, Jensen RH, et al. Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: Data from a nationwide fungaemia surveillance programme. *Clin Microbiol Infect*. 2013; 19(8):E343–53. Recent data from the largest nation-wide (and thus population based) ongoing surveillance programme of fungal blood stream infections.
 20. Shields RK, Nguyen MH, Press EG, et al. Anidulafungin and

- micafungin minimum inhibitory concentration breakpoints are superior to caspofungin for identifying FKS mutant *Candida glabrata* and echinocandin resistance. *Antimicrob Agents Chemother.* 2013 Sep 23;57:6361–5. First study showing that anidulafungin and micafungin can be used as markers for caspofungin susceptibility for CLSI testing.
21. Arendrup MC, Pfaller MA. Caspofungin Etest susceptibility testing of *Candida* species: risk of misclassification of susceptible isolates of *C. glabrata* and *C. krusei* when adopting the revised CLSI caspofungin breakpoints. *Antimicrob Agents Chemother.* 2012 Jul; 56(7):3965–8.
 22. Eschenauer GA, Nguyen MH, Shoham S, et al. Real-World Experience with Echinocandin MICs against *Candida* Species in a Multicenter Study of Hospitals That Routinely Perform Susceptibility Testing of Bloodstream Isolates. *Antimicrobial Agents and Chemotherapy.* 2014 Apr 1; 58(4):1897–906. Comprehensive multicentre study on the performance of Sensititre Yeast One for routine susceptibility testing of *Candida* and echinocandins. A nice interlaboratory agreement was documented, but the adoption of the revised CLSI breakpoints for *C. glabrata* and *C. krusei* resulted in an unacceptably high number of misclassifications of susceptible isolates as intermediate or resistant.
 23. Pfaller MA, Diekema DJ, Jones RN, Castanheira M. Use of Anidulafungin as a Surrogate Marker to Predict Susceptibility and Resistance to Caspofungin among 4,290 Clinical Isolates of *Candida* using CLSI Methods and Interpretive Criteria. *Journal of Clinical Microbiology.* 2014 Jun 20. Comprehensive evaluation of the performance of anidulafungin as marker for caspofungin resistance using the CLSI method.
 24. Pfaller MA, Messer SA, Diekema DJ, et al. Use of Micafungin as a Surrogate Marker To Predict Susceptibility and Resistance to Caspofungin among 3,764 Clinical Isolates of *Candida* by Use of CLSI Methods and Interpretive Criteria. *Journal of Clinical Microbiology.* 2014 Jan 1; 52(1): 108–14. Comprehensive evaluation of the performance of micafungin as marker for caspofungin resistance using the CLSI method.
 25. Perlin DS. Resistance to echinocandin-class antifungal drugs. *Drug Resistance Updates.* 2007;10:121–130.
 26. Perlin DS. Echinocandin-resistant *Candida*: molecular methods and phenotypes. *Curr Fungal Infect Rep.* 2011;5:113–119
 27. Johnson ME, Katiyar SK, Edlind TD. A new Fks hotspot for acquired echinocandin resistance in yeast, and its contribution to intrinsic resistance of *Scedosporium* species. *Antimicrobial Agents*

- and Chemotherapy. 2011;55:3774–3781.
28. Katiyar S, Pfaller M, Edlind T. *Candida albicans* and *Candida glabrata* clinical isolates exhibiting reduced echinocandin susceptibility. *Antimicrobial Agents and Chemotherapy*. 2006;50:2892–2894.
29. Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol*. 2011;6:441–457.
30. Garcia-Effron G, et al. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrobial Agents and Chemotherapy*. 2009;53:3690–3699.
31. Lackner M, et al. Position and numbers of FKS mutations in *C. albicans* selectively influence in vitro and in vivo susceptibility to echinocandin treatment. *Antimicrobial Agents and Chemotherapy*. 2014; 58:3626–3635.
32. Shields RK, et al. The presence of an FKS mutation rather than MIC is an independent risk factor for failure of echinocandin therapy among patients with invasive candidiasis due to *Candida glabrata*. *Antimicrobial Agents and Chemotherapy*. 2012; 56:4862–4869. [
33. 36. Castanheira M, et al. Frequency of fks mutations among *Candida glabrata* isolates from a 10-year global collection of bloodstream infection isolates. *Antimicrobial Agents and Chemotherapy*. 2014;58:577–580
34. 37. Howard SJ, Lestner JM, Sharp A, Gregson L, Goodwin J, Slater J, Majithiya JB, Warn PA, Hope WW. 2011. Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: Clinical implications for antifungal therapy. *J Infect Dis* 203: 1324–1332.
35. 38. Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W. 2012. Differential in vivo activity of anidulafungin, caspofungin and micafungin against *Candida glabrata* with and without *FKS* resistance mutations. *Antimicrob Agents Chemother* 56: 2435–2442.
36. 40. Katiyar SK, et al. Fks1 and Fks2 are functionally redundant but differentially regulated in *Candida glabrata*: implications for echinocandin resistance. *Antimicrobial Agents and Chemotherapy*. 2012; 56:6304–6309.
37. 41. Pasquale T, et al. Emergence of *Candida tropicalis* resistant to

- casposfungin. *J Antimicrob Chemother.* 2008; 61:219.
- 38.42. Fekkar A, et al. Rapid emergence of echinocandin resistance during *Candida kefyr* fungemia treatment with casposfungin. *Antimicrobial Agents and Chemotherapy.* 2013;57:2380–2382.
- 39.43. Jensen RH, Johansen HK, Arendrup MC. Stepwise development of a homozygous S80P substitution in Fks1p, conferring echinocandin resistance in *Candida tropicalis*. *Antimicrobial Agents and Chemotherapy.* 2013;57:614–617.
- 40.44. Pfaller MA, et al. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. *Journal of Clinical Microbiology.* 2010;48:52–56.
- 41.45. Tortorano AM, et al. A 1-year prospective survey of candidemia in Italy and changing epidemiology over one decade. *Infection.* 2013;41:655–662
- 42.46. Pfaller MA, et al. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, casposfungin, and micafungin: six years of global surveillance. *J Clin Microbiol.* 2008;46:150–156.
- 43.47. Ghannoum MA, et al. Differential in vitro activity of anidulafungin, casposfungin and micafungin against *Candida parapsilosis* isolates recovered from a burn unit. *Clin Microbiol Infect.* 2009;15:274–279.
- 44.48. Forrest GN, Weekes E, Johnson JK. Increasing incidence of *Candida parapsilosis* candidemia with casposfungin usage. *J Infect.* 2008;56:126–129.
- 45.49. Garcia-Effron G, et al. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* accounts for reduced echinocandin susceptibility. *Antimicrob Agents Chemother.* 2008;52:2305–2312.
- 46.50. Johnson ME, Katiyar SK, Edlind TD. New Fks hot spot for acquired echinocandin resistance in *Saccharomyces cerevisiae* and its contribution to intrinsic resistance of *Scedosporium* species. *Antimicrobial Agents and Chemotherapy.* 2011;55:3774–3781.
47. Lewis KIM. Riddle of biofilm resistance. *Antimicrob Agents Chemother* (2001) 45: 999-1007. Doi:10.1128/AAC.45.4.999-1007.2001
48. Donlan RM. Biofilm and device - associated infections. *Emerg Infect Dis* (2001) 7:277-81. doi: 10.3201/eido702.010226.
- 49.51. d’Enfert, C., 2009. Hidden killers: persistence of opportunistic fungal pathogens in the human host. *Curr. Opin. Microbiol.*, Epub

ahead of print.

- 50.52. Desai JV, et al. Regulatory role of glycerol in *Candida albicans* biofilm formation. *MBio*. 2013; 4:e00637–00612.
- 51.53. Munro CA, Selvaggini S, de Bruijn I, Walker L, Lenardon MD, Gerssen B, Milne S, Brown AJ, Gow NA. 2007. The PKC, HOG and Ca²⁺ signalling pathways co-ordinately regulate chitin synthesis in *Candida albicans*. *Mol Microbiol* 63: 1399–1413
- 52.54. Healey KR, Katiyar SK, Castanheira M, Pfaller MA, Edlind TD. 2011. *Candida glabrata* mutants demonstrating paradoxical reduced caspofungin susceptibility but increased micafungin susceptibility. *Antimicrob Agents Chemother* 55: 3947–3949
53. M. Ponomarenko, N. Kolchanov, in *Brenner's Encyclopedia of Genetics (Second Edition)*, 2013
- 54.55. Cowen LE, Lindquist S. 2005. Hsp90 potentiates the rapid evolution of new traits: Drug resistance in diverse fungi. *Science* 309: 2185–2189.
- 55.56. Singh-Babak SD, Babak T, Diezmann S, Hill JA, Xie JL, Chen YL, Poutanen SM, Rennie RP, Heitman J, Cowen LE. 2012. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. *PLoS Pathog* 8: e1002718.
- 56.57. LaFayette SL, Collins C, Zaas AK, Schell WA, Betancourt-Quiroz M, Gunatilaka AA, Perfect JR, Cowen LE. 2010. PKC signaling regulates drug resistance of the fungal pathogen *Candida albicans* via circuitry comprised of Mkc1, calcineurin, and Hsp90. *PLoS Pathog* 6: e1001069
- 57.58. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* 2002; 347: 2020–9
- 58.59. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; 347:408–15
- 59.60. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. *N Engl J Med* 1997; 337:15–21.
- 60.61. Farmakiotis, D.; Tarrand, J.J.; Kontoyiannis, D.P. Drug-resistant *Candida glabrata* infection in cancer patients. *Emerg. Infect. Dis.* 2014, 20, 1833–1840
- 61.62. Alexander, B.D.; Johnson, M.D.; Pfeiffer, C.D.; Jimenez-

Ortigosa, C.; Catania, J.; Booker, R.; Castanheira, M.; Messer, S.A.; Perlin, D.S.; Pfaller, M.A. Increasing echinocandin resistance in *Candida glabrata*: Clinical failure correlates with presence of *FKS* mutations and elevated minimum inhibitory concentrations. *Clin. Infect. Dis.* **2013**, *56*, 1724–1732.

62.63. Kullberg BJ, Arendrup MC. Invasive candidiasis. *N Engl J Med* **2015**; *373*:1445–56.

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