Emergence of antifungal resistance and the promise of new antifungal agents

29th ECCMID, Amsterdam/Netherlands
13 – 16 April 2019

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Roadmap

- Emergence of antifungal resistance
- Drugs available
- New drugs in pipeline
Invasive candidiasis

- Drug resistance develops in pathogens
  - *C. glabrata* and *C. auris*
  - *Rhodotorula rubra* – R to echinocandins
- Requires early-stage treatment
- Colonisation versus infection (?)
Invasive and chronic aspergillosis

- Increasing resistance to azoles in *A. fumigatus* and cryptic species & other *Aspergilli*
- Early treatment or prevention is essential
- Shift to new “hosts”
Mucormycosis

- Poor prognosis
- Selection under *Aspergillus*-covering drugs
- No or only few diagnostic tests
Disseminated cryptococcosis

✔ Even with ART, there are still many new cases
✔ No new therapies in more than 25 years

© by author
Dimorphic mycoses

- Geographically restricted
- Infect both immunocompetent and immunosuppressed pts
- A vaccine would be welcomed and is in development
Other fungi

- Lomentospora (Scedosporium) prolificans
- Paecilomyces lilacinus-R to amphotericin B and itraconazole
  - difficult to treat
  - worse outcome
The polyenes: broadest drugs, side-effects & dosages are different, some pts tolerate others not, resistance is rarely acquired, most fungi are primary resistant, iv only,…

The azoles: broad and small spectrum drugs, side-effects & dosages are different, iv and os, resistance induction is high, depends from drug to drug, cross resistance, multiple resistance mechanisms, …

The echinocandins: focus on Candida, side-effects are low, dosages are different, iv, resistance induction is moderate, cross resistance (?), one fits all (?), resistance based mutations in FKS1, most prominently in C. glabrata, …

The rest: fucytosin (reserve?), strong resistance induction in monotherapy, small coverage,…….
• 1<sup>st</sup>, still high mortality, also including “susceptible” strains.
• 2<sup>nd</sup>, need of rapid fungicidal activity, treatment too long.
• 3<sup>rd</sup>, need to widen the spectrum, primarily drug-resistant fungi.
• 4<sup>th</sup>, need to optimize combination of agents.
• 5<sup>th</sup>, increase of sec. resistant strains, azoles & echinocandins.
• 6<sup>th</sup>, safety remains a very important issue.
• 7<sup>th</sup>, four antifungal classes.
• 8<sup>th</sup>, only few oral drugs with broader spectrum.
• 9<sup>th</sup>, need to optimize compliance (once weekly dosing).

Phase I  Testing of drug on healthy volunteers for safety
Phase II  Testing of drug on patients to assess efficacy and side effects, therapeutic dose
Phase III Testing of drug on patients to assess efficacy, effectiveness and safety
- Improving existing antifungals
- New agents in development
- Novel pathways and targets
- Repurposing old drugs
- Host immune-cell targeted approaches
- Antifungal biological agents
Antifungal targets
New agents – near to or in clinic

SYSTEMIC
• Acrylamide (Toyama; T-2307)
• Inhibitor of fungal glycosylphosphatidylinositol biosynthesis (Amplyx; APX001A)
• Novel and as-yet-unknown targets (Vical; ASP 2397) Discontinue!
• Orotomide (F2G; F901318, olorofim)
• Novel CYP inhibitor (Viamet; VT1129, quilseconazole)
• Novel CYP inhibitor (Mycovia; VT1161)
• New echinocandin (Cidara; CD101, Rezafungin)
• Oral glucan synthase inhibitor (Scynexis, SCY-078, Ibrexafungerp)
• New formulations of amphotericin B (MAT2203, Matinas; cochleate, AMB self-emulsifying drug delivery system iCo)

INHALED
• Itraconazole (Pulmatrix, PUR1900), start phase 2
• Novel triazole antifungal (Pulmocide; PC945, anti-Aspergillus)
<table>
<thead>
<tr>
<th>New Drugs</th>
<th>Candida</th>
<th>Aspergillus</th>
<th>Mucorales</th>
<th>Scedosporium</th>
<th>Fusarium</th>
<th>C. neoformans</th>
<th>Trichosporon</th>
<th>Dimorphic fungi</th>
<th>Dermatophytes</th>
<th>Black fungi</th>
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</thead>
<tbody>
<tr>
<td>Nikkomyzin Z</td>
<td></td>
<td>✓</td>
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<tr>
<td>APX001A, E1210</td>
<td>+ C. auris - C. krusei</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>AR-12 (Arno Therapeutics)</td>
<td>C. albicans Non-C. albicans + Azole-R + AmphiB-R</td>
<td>✓</td>
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<tr>
<td>ASP2397 (Vical) VL2397</td>
<td>C. glabrata Echinocandin-R</td>
<td>✓</td>
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<tr>
<td>F901318 (Olorofim) F2G</td>
<td>+ AmphiB-R + Azole-R</td>
<td>✓</td>
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<td>T-2307 (Toyama)</td>
<td>✓</td>
<td>✓ (R)</td>
<td>✓</td>
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<tr>
<td>CD101 (Cidara) Biafungin, Rezafungin + Caspo-R</td>
<td>✓</td>
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<tr>
<td>SCY-078/MK-3118 (oral/iv)</td>
<td>✓</td>
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<tr>
<td>VT-1161 (Viamet)</td>
<td>✓</td>
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<tr>
<td>VT-1598 (Viamet)</td>
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<td>VT-1129</td>
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<tr>
<td>MATT2203, oAMB</td>
<td>✓</td>
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</table>
Streptomyces is the largest genus of the Actinobacteria with over 500 known species.
They are found predominantly in soil and decaying vegetation.
They are gram positive and have a mycelium-type growth resembling molds.
**Amphotericin B** obtained from *S. nodosus*.
First-line treatment of systemic fungal infections.
Number limitations: toxicity, iv, ....
Strategies to improve oral bioavailability of formulations

- Use of co-solvents
- Cyclodextrins
- Salt formation
- Solid dispersions
- Nano- and Micro suspensions
- Use of surfactants

Lipid based formulations

- Liposomes
- SMEDDS
- Solid lipid nanoparticles
- Microemulsions
- Polymeric nanoparticles

Prodrug
Amphotericin B (Low solubility, High molecular weight, Higher systemic toxicity)

Nanotechnology Approaches

- Liposomes (Reduced nephrotoxicity, high cost, poor stability)
- Nanoemulsion
- Nanoparticles
- Ultradeformable liposomes
- Dendrimers
- Carbon nanotubes
- Ethosomes
- Nanospheres
- Polymerosomes
<table>
<thead>
<tr>
<th>14 AmB oral formulation</th>
<th>Efficacy</th>
<th>Stability</th>
</tr>
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<tbody>
<tr>
<td>Solid lipid nanoparticle [47]</td>
<td>Lower kidney tissue concentration, 105% Fo of Fungizone&lt;sup&gt;®&lt;/sup&gt;</td>
<td>2-8 °C for 3 months, 15 days ≥ 25 °C</td>
</tr>
<tr>
<td>PLGA–PEG nanoparticle [43,48]</td>
<td>Increase antifungal activity 4-fold in vitro</td>
<td>N/A</td>
</tr>
<tr>
<td>Chitosan-coated nanostructured lipid carriers [42]</td>
<td>N/A</td>
<td>63.9% AmB retained encapsulated after 30 min incubation in SIF</td>
</tr>
<tr>
<td>Lecithin-based mixed polymeric micelles [49]</td>
<td>Less toxic in HT29 cells 150% Fo of Fungizone&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Increase solubility</td>
</tr>
<tr>
<td>O/W microemulsion [50]</td>
<td>Slightly less toxic than free DMSO</td>
<td>Increase the solubility by 1000 folds</td>
</tr>
<tr>
<td>Pickering emulsion [51]</td>
<td>N/A</td>
<td>Stable one month under refrigeration</td>
</tr>
<tr>
<td>Tragacanth/ acrylic acid copolymer [52]</td>
<td>No mortality observed in mice comparing with free AmB Improve oral bioavailability comparing with free AmB</td>
<td>N/A</td>
</tr>
<tr>
<td>Chitosan and porphyrin polymeric nanocarrier [53]</td>
<td>23-fold antifungal activity than Ambisome&lt;sup&gt;®&lt;/sup&gt; Slightly less toxic than Fungizone&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Less degradation in SIF and a superior release profile for up to 12 h</td>
</tr>
<tr>
<td>Chitosan–EDTA microparticles [54]</td>
<td>N/A</td>
<td>12-fold improvement in in vitro dissolution relative to pure AmB</td>
</tr>
<tr>
<td>Carbon Nanotubes [55,56]</td>
<td>Inhibit the parasite load in a dose-dependent manner No evidence of toxicity in mice and hamster models</td>
<td>N/A</td>
</tr>
<tr>
<td>Cubosomes (cubic liquid crystal nanoparticles) [57]</td>
<td>low dose of AmB-loaded cubosomes shows low kidney concentration than Fungizone&lt;sup&gt;®&lt;/sup&gt; 285% bioavailability of Fungizone&lt;sup&gt;®&lt;/sup&gt;</td>
<td>74% detectable AmB after 3 h in SIF</td>
</tr>
<tr>
<td>GCPQ nanoparticles [58]</td>
<td>Absolute Fo is 24.7% Higher concentration in liver, lung and spleen</td>
<td>Stable for a year on storage</td>
</tr>
<tr>
<td>Coacate–CAMB/MAT2203 [59,60]</td>
<td>100% survival comparing with Fungizone&lt;sup&gt;®&lt;/sup&gt; and Ambisome&lt;sup&gt;®&lt;/sup&gt; No serious adverse event in Phase I study</td>
<td>Stable for 4 months at 4 °C</td>
</tr>
<tr>
<td>SEDDS (iCo-010/019) [66]</td>
<td>&lt;99% reduction in parasitic infection in a murine model 95% inhibition when compared to control</td>
<td>&gt;75% over 60 days in 30 °C; &gt;95% after 4 h in SIF</td>
</tr>
</tbody>
</table>

Abbreviations: SIF, simulated intestinal fluid; Fo, oral bioavailability.
Self-emulsifying drug delivery systems are mixtures of oil and surfactants, +/-co-solvent or co-emulsifiers, which emulsify under conditions of gentle agitation, similar to those in the gastrointestinal tract.

SEDDS is considered to be the broad term referring to emulsions producing a droplet size, ranging from nanometers to microns.
1. Protection against enzymatic degradation
2. Mucus-penetrating oil droplets
3. Transcytosis-based transcellular transport
4. Surfactant-mediated enhancement of paracellular transport

Cytoplasm
Endosome
Delivery of protein to its cellular destination

Mucus layer with low enzymatic activity

Amphophilic Protein Surfactant Carrier Therapeutic Molecule
iCo Therapeutics Announces Additional Positive Pharmacokinetic Results and Presentation at Global Investment Conference

September 6, 2018, Vancouver, Canada — iCo Therapeutics (“iCo” or the “Company”) (TSX-V: ICO) (OTCQB: ICOTF), today announced additional positive pharmacokinetic data from its recent Phase 1 study of its Oral Amphotericin B (Oral Amp B) candidate. Previously the Company reported that Oral Amp B achieved a median Cmax of 28 ng/mL and AUC0-inf of 1030 hr*ng/mL at the lowest dose of Oral Amphotericin B of 100 mg, demonstrating superiority of area under the concentration time curve from time zero to infinity, when compared to published 200 mg, 400 mg and 800 mg oral cochleate formulation data by the closest competitor. Today iCo reported a median AUC0-inf of 2029 hr*ng/mL at the 400 mg dose of Oral Amphotericin B, representing an approximate doubling of the critical AUC0-inf measure at an increased dose. The Company intends to study both the 100 mg and 400 mg dose in the next clinical study. In its Phase 1 study 100 mg, 200 mg, 400 mg and 800 mg doses were studied.

Stated Dr. Peter Hnik “The prolonged plasma half-life and increased AUC as a function of dose suggests that the oral Amphotericin B formulation has a long circulation time which may result in the ability of the formulation to increase Amphotericin B tissue concentrations within infected tissues without the associated GI, liver and kidney toxicity”.
**AmB cochleates** (MAT 2203, cochleat AMB, Matinas)

- Encapsulating of drug in a "roll" of lipid, cAMP
- Taken up by cells in the GI tract and gives tissue levels above MIC of *Candida* but low serum levels
- Reduced toxicity, targeted delivery, oral delivery
- Phase I trial – well tolerated with no adverse events
- Phase IIa trial ongoing for mucutaneous candidiasis refractory to other oral antifungals
- Phase 2: vulvovaginitis
  - cAMP: 36% cure
  - FLU: 81% cure

This system is lipid-based technology comprising negatively charged phospholipid bilayer as a sheet rolled up in a cigar like spiral roll via interaction between bilayer and multivalent counter ions (bridging agents).
✓ **SUBA** technology: enhancing the bioavailability of poorly soluble drugs.
✓ Increase absorption of drugs in the GI to achieve “super bioavailability” compared to conventional formulations.
✓ Spray drying technology to create nanoparticles (<40μm) of itraconazole in a polymer matrix = increased solubility.
✓ Itraconazole vs SUBA®-itraconazole (44% vs 81% reached \( C_{\text{trough}} \) mean level of >1000 ng/mL) & \( C_{\text{trough}} \) 1200ng/ml vs 1600ng/ml).
✓ Immunocompromised and non-immunocompromised:
  - Blastomycosis, Histoplasmosis, Aspergillosis

- Increased bioavailability
- Reduced intra/inter-patient variability
- Reduced side effects

Itraconazole drug substance prior to processing
SUBA™ Itraconazole solid dispersions containing 40% Itraconazole
A prospective, multi-center, randomized, open-label parallel arm study involving patients with proven or probable invasive endemic fungal infection to ascertain the pharmacokinetics, safety, efficacy, tolerability and health economics of oral SUBA-itraconazole compared to conventional itraconazole. Patients will receive SUBA-itraconazole or conventional itraconazole over a 42 day period and then continue therapy until Day 180.
**F901318 (F2G, olorofim)**

- First compound in the orotomide class
- Dihydroorotate dehydrogenase inhibitor = trimethoprim-like mechanisms (DHOH involved in pyrimidine biosynthesis)
- Humans have this enzyme, but > 2000-fold difference in inhibitory concentration between human and fungal enzymes
- Is a DHOH inhibitor of *Aspergillus* sp. incl. *terreus, flavus, niger, tanneri, nidulans*
- In phase 2 development as iv and oral agent
- Activity against *Aspergillus* (R- Azoles & amB), *Fusarium* & *Scedosporium* in in-vivo models
- No activity against *Candida* or *Mucorales*
- Phase 3
  - Refractory aspergillosis
  - Scedosporiosis
  - Lomentosporiosis

Nat Rev Drug Discov 16:603-616, 2017
JID 2017; 216:S3
JMedChem2018, 61:5484
APX001 (Amplyx)

- APX001 (prodrug) alkaline phosphatase
- APX001A active moiety
- APX001A inhibits Gwt1 enzyme
- Gwt1 is an early step in glyosylphosphatidylinositol (GPI) -anchor biosynthesis
- Gwt1 essential for anchoring mannoprotrines in cell wall: inhibits fungal growth
- Activity in vitro against Aspergillus (R-Azo&AmB) Candida, C. auris, Mucor, Scedosporium, Cryptococcus and Coccidioidomycosis
- Less active against C. krusei
- No human analog!
- Phase 1 trials completed (IV & oral)
- Phase 2 (IC, IA & rare molds)
- ORPHAN drug designation for 6 indications
  - IC, IA, Coccidioidomycosis, Scedosporium, Fusarium, Mucorales and FDA: Cryptococcus

Inositol acyltransferase inhibitor
APX2039 (active moiety of APX2096 prodrug) is 32-fold more potent than APX001A versus Cryptococcus, but less active vs C. albicans and A. fumigatus
Acrylamide: T-2307 (Toyama)

- Member of a class of aromatic diamidines
- **Novel mechanism of action** - selective transportation into fungal cells and inhibition of mitochondrial membrane potential
- Similar to pentamidine Phase 1
- Reported to have activity against *Fusarium*
- In vitro & in vivo fungicidal activity against broad spectrum yeasts and moulds (*A. fumigatus (R-Azol)* & Pneumocystis (*& P. falciparum*)

Mitsuyama et al. AAC 2008, 52:1318
Rezafungin  (CD101, Cidara, Biafungin)

- Structural modification yields chemical stability & enhanced biological properties
- IV echinocandin with long half life
- AUC compared to anidulafungin
  ~ 2.5 times higher during first 2d
  ~ 1.6 times higher during first 7d
- Once weekly dosing
- Covers *Candida (R-Ech), Aspergillus, Trichophyton mentagrophytes, rubrum, and Microsporum gypseum*
- Phase II trial in invasive candidiasis completed (Restore), superior over micafungin
- Prophylaxis trial in alloHSCT (ReSPECT)
MK-3118/SCY-078 (Ibrexafungerp, Scynexis)

- First in class triterpenoid, novel class of β-glucan synthase inhibitor
- Likely acts at a different site than echinocandins
- Oral glucan synthase inhibitor
- Low risk of interactions with CYP enzymes
- Activity against Aspergillus (azole R) and Candida in vitro and preclinical models, incl. Pneumocytis
- Phase I study reported, oral, iv
- Phase 2/3: invasive C, vulvovag. C (74% for SCY vs 60% for FLU), IA combo + azoles, refractory IFI, C. auris
VT1129  (Viamet, Quilseconazole)

- Phase 1 – cryptococcal meninigits
- Novel CYP inhibitor (metalloenzyme blockers)
- Demonstrates potent in vitro activity against C. neoformans and C. gattii.
- Strong in vitro activity against C. glabrata and C. krusei resistant to azole and echinocandin antifungals

VT1161  (Mycovia)

- Phase 3 – recurrent VVC
- Novel CYP inhibitor (orally-administered inhibitor)
- completed Phase 2b trials for the treatment of onychomycosis, or fungal nail infection, and recurrent vulvovaginal candidiasis (RVVC): the recurrence rate of one or more vulvovaginal candidiasis episodes in the placebo arm was 66% while the four VT-1161 arms ranged from 0-11%.
- VT-1161 displayed potent activity against a range of Candida species including C. glabrata.
ASP-2397 (Vical)

- Natural product from *Acremonium* (leaf-litter fungus)
- Active transport into *A. fumigatus* occurs via iron siderophore transporter Sit1
- Intracellular target unknown
- Activity against *Candida* (R-Ech) and *Aspergillus*
- Low propensity for CYP interactions
- Phase II trials of VL2397 for primary IA therapy
- **Phase 2 – DISCONTINUE CLINICAL DEVELOPMENT in Feb 2019**
✓ **Calcineurin**  
Calcineurin pathway: stress response in fungi; essential for virulence in *C. albicans* and *A. fumigatus*, linked to HSP90

✓ **Spingolipid synthesis**  
Research from cancer, important for cellular metabolism

✓ **Trehalose**  
Trehalose pathway is important for glycolysis and regulates sugar trehalose,

✓ **Others**  
HOG pathways  
MAP kinase pathways  
RAS ......
Cloudbreak molecules (Cidara)

- Hybrid molecules/Immunotherapy
- Fungal binding targeting molecule fused to an immune effector domain
- Links antifungal (such as AmB) with immune activator (such as Fc portion of antibody)
- Promising data with gram negative bacteria
- Efficacy in vitro, ex vivo and in animal models

Direct kill: TM (target moiety) tightly binds conserved targets to kill fungi
Immunomodulatory: EM (effector moiety, antibody) recruits and initiates an innate immune system response

Nat Rev Drug Discov 16:603-616, 2017
JID 2017; 216:S3
JMedChem2018, 61:5484
Antifungal compounds with novel targets

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular target</th>
<th>Target species or relevant disease</th>
<th>Development stage</th>
</tr>
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</table>
| Cyclosporine, tacrolimus or rapamycin | mTOR or calcineurin | • Broad spectrum  
  • To be used alongside existing antifungals | Approved for post-transplant immunosuppression        |
| Rifampicin                        | RNA polymerase         | • Broad spectrum  
  • To be used alongside existing antifungals | Approved for bacterial infections                     |
| Sertraline                        | Serotonin reuptake     | • Cryptococcal meningitis  
  • To be used alongside existing antifungals       | Approved for depression                               |
| Tamoxifen                         | Oestrogen receptor     | • Cryptococcus  
  • To be used alongside existing antifungals      | Approved for oestrogen receptor-positive breast cancer |
| Verapamil                         | Calcium channel        | • Broad spectrum  
  • To be used alongside existing antifungals      | Approved for cardiac conditions (including hypertension and arrhythmias) |

Adjunctive Sertraline for treatment of HIV-associated cryptococcal meningitis (ASTRO-CM)

Trial stopped after enrolling 460 patients:
18 week mortality: 52% sertraline and 46% in placebo

Rhein et al. CROI 2018
Concluding remarks

- There are plenty of new drugs in the pipeline with good in vitro data, against S and R strains
- Oral availability, reduced toxicity
- Substances cover emerging or rare pathogens
- Some drugs act very specific, attack different critical targets
- Some new drugs are superior, similar or worse when compared to control
- Clinical challenge: you need to know the fungal pathogen for a targeted treatment
- Need more clinical studies regarding efficacy
- Some new drugs promise a new horizon