Challenges and Lessons Learned Developing DAV132, a Novel Therapy Protecting Gut Microbiota from Antibiotic-Induced Dysbiosis

Florence Séjourné, CEO of Da Volterra
Founder & Board Member of the BEAM Alliance
florence.sejourne@davolterra.com

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All Antibiotics Provoke Intestinal Microbiota Dysbiosis

During oral and parenteral antibiotic treatments, antibiotic residues reach the colon where they kill numerous bacteria and provoke a strong dysbiosis.

**Healthy Microbiota**
High diversity:
- Beneficial to health,
- Symbiosis with human body functions

**Disrupted Microbiota**
Strongly decreased diversity, i.e. ‘dysbiosis’ with human body:
- Dysfunctional gut barrier and colonization by pathogenic bacteria such as *C. difficile*
- Multiplication of resistant clones
- Degraded immunity and immune response
- Oxidative stress increase
- Altered metabolism

Adapted from Jernberg C et al. Microbiology. 2010
Concept - Gut Microbiota Protective Therapies

Maintaining the Healthy Microbiota Function by Preventing Antibiotic-Induced Intestinal Disruption and Consequences

- **HEALTHY STATE**: High diversity
- **DYSBIOSIS STATE**: Low diversity
- **HEALTHY STATE**: High diversity

Antibiotics i.v. and oral

Antibiotics i.v. and oral + Gut Microbiota Protective Therapy

Expected benefits in patients receiving antibiotics
- Prevent *C. difficile* infections in patients at risk
- Limit emergence and dissemination of resistant strains
- Increase efficacy of immunity-based cancer treatments
**DAV132 Mode of Action: Capturing Antibiotic Residues in the Colon**

1. **DAV132** is administered orally with antibiotics. The antibiotics reach the bloodstream from the upper gastrointestinal tract without interference with DAV132, whose coating remains intact.

2. DAV132’s coating opens in the colon and the adsorbent irreversibly captures (adsorbs) the antibiotic residues.

3. Antibiotic residues bind to DAV132 and are eliminated in the patients' stools.

**No interference with systemic absorption and efficacy of co-administered antibiotics**

**Inactivation of the antibiotic residues in the caecum and colon**

**Microbiota protected from antibiotic-induced disruption**
DAV132 – Development Status

✓ Non-Clinical: Numerous preclinical proof of concept data on prevention of CDI in Hamster models + Breakthrough proof of concept in immuno-oncology

✓ Clinical: Data generated in 496 individuals exposed to DAV132 in six Phase 1 and one Phase 2 clinical trials completed so far

✓ Demonstrated efficient and reproducible mode of action in humans:
  ▪ Free antibiotic fecal concentrations decreased by 99% with DAV132
  ▪ No impact of DAV132 on the plasmatic concentration and efficacy of concomitant drugs taken by the patients
  ▪ Very good protection of the intestinal microbiota diversity and functional colonization by C. diff

✓ Good safety profile, even in severely-ill patients

✓ CMC: Product composition, process and characterization complete for Phase 3-stage
DAV132 Efficiently Captures Antibiotics Without Impacting their Plasma Concentrations

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Antibiotic</th>
<th>Adsorption (\text{in vitro}(%))</th>
<th>Adsorption (\text{ex vivo}(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Amoxicillin</td>
<td>&gt; 99</td>
<td>&gt; 99</td>
</tr>
<tr>
<td></td>
<td>Piperacillin</td>
<td>&gt; 99</td>
<td>&gt; 98</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Cefalexin</td>
<td>&gt; 98</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Cefazolin</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cefdinir</td>
<td>&gt; 99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cefixime</td>
<td>&gt; 99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>&gt; 98</td>
<td>&gt; 99</td>
</tr>
<tr>
<td></td>
<td>Cefpodoxime</td>
<td>&gt; 99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>&gt; 99</td>
<td>&gt; 96</td>
</tr>
<tr>
<td></td>
<td>Cefepime</td>
<td>&gt; 98</td>
<td>98</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Ertapenem</td>
<td>&gt; 99</td>
<td>&gt; 99</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>&gt; 97</td>
<td>&gt; 99</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>&gt; 97</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>Ciprofloxacin</td>
<td>&gt; 99</td>
<td>&gt; 99</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin</td>
<td>&gt; 99</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td>&gt; 99</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Lomefloxacin</td>
<td>&gt; 99</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>Lincosamide</td>
<td>Clindamycin</td>
<td>&gt; 95</td>
<td>97</td>
</tr>
<tr>
<td>Macrolide</td>
<td>Azithromycin</td>
<td>&gt; 99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarithromycin</td>
<td>&gt; 99</td>
<td></td>
</tr>
</tbody>
</table>

In vitro & Ex vivo experiments
85 antibiotics tested

DAV132 efficiently captures antibiotics in the colon

DAV132 does not impact the plasma PK of oral and IV antibiotics

Sources: de Gunzburg et al. JID 2018; Vehreschild, et al. JAC. 2022
DAV132 Preserves the Gut Ecosystem from Dysbiosis

**With Fluoroquinolones**

<table>
<thead>
<tr>
<th></th>
<th>Day 6</th>
<th>End of ABX</th>
<th>10 ± 1 days after end of ABX</th>
<th>30 ± 2 days after end of ABX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p</strong></td>
<td>0.006</td>
<td>0.03</td>
<td>0.52</td>
<td>0.58</td>
</tr>
</tbody>
</table>

The Shannon diversity index is commonly used to characterize species diversity in a community. It accounts for both abundance and evenness of the species present.

**Sources:** Harris et al. AAC. 2020; Mark Wilcox Lab; Vehreschild, et al. JAC. 2022
DAV132 Maintains the Functions of the Microbiota

Resistance to Colonization by *C. diff*

**Design: Colonization Resistance Assay for *C. diff* in Stool (CRACS)**

Patient + ABX ± DAV132

Stools C. diff suspension

Measure of C. diff proliferation for 3 days

**Phase 1 Clinical Trial**
Healthy Volunteers

**Phase 2 Clinical Trial**
Patients

**Sources:** Harris et al. AAC. 2020; Mark Wilcox Lab; Vehreschild, et al. JAC. 2022; Unpublished data
DAV132 Significantly Reduces the Counts of Vancomycin-Resistant Enterococci (VRE) at the End of FQ Antibiotics Treatment

Phase 2 Clinical Trial: VRE counts at End-of-FQ treatment are reduced in the feces of patients treated with DAV132 (not carriers at Day 1)

Sources: Vehreschild, et al. JAC. 2022
Phase 3 Design for DAV132 to Demonstrate the Prevention of CDI in an Enriched Patient Population (AML)

- A multicenter, randomized, placebo-controlled, parallel-arm clinical trial (phase III)
- To assess the efficacy of DAV132, compared to placebo, in preventing *C. difficile* infection in newly diagnosed AML or high-risk MDS patients treated with intensive chemotherapy

**Primary endpoint:** occurrence of CDI during the 120 days following randomization
Lessons Learned – Regulatory and Clinical Operations

• Primary analysis: competing risks analysis with CDI occurrence as the event of interest and death as a competing risk, with cause-specific hazard ratio as a statistical outcome, “Time to CDI” being the variable of interest on which the competing risks approach is based.
  ✓ Cause-specific hazard ratio and cumulative incidence do not focus on the time to CDI but they express the actual risk of developing a CDI at any point in time in the study, thus informative on the incidence of CDI.
  ✓ Simulations performed that showed that the competing risks approach does not lead to over-conclude in the unlikely case DAV132 only delays (but not prevents) CDI occurrence.
  ✓ Approach developed in partnership with experts from STAT-NET as appropriate to conclude on a meaningful clinical benefit.
  ✓ Sample size re-estimation planned with 2 interim analyses to optimize futility decision*.

• Study initiated in Europe as a public-private partnership in COMBACTE-NET but which had to stop in July 2022 for operational futility (not enough sites, low recruitment rate)

* Marlieke de Kraker, Which design aspects can enhance clinical trial efficiency? ECCMID Educational Workshop 2022
Low-Diversity Microbiota is a Biological Marker Correlated to CDI Risk

Correlation between low-diversity microbiota (alpha-diversity Shannon Index) and occurrence of *C. difficile* infections (CDI) has been validated in the scientific literature:

**Powerful Predictor for *C. difficile* Infection Severity in Hamsters**

![Graph showing the correlation between Shannon index change and mortality in hamsters.](image)

*C. diff* mortality increases when Shannon decreases

Logistic models of mortality according to the change of Shannon index between D0 and D3 after pooling data from antibiotic-treated animals.


**A lower microbiota diversity is predictive of CDI**

![Box plots comparing Shannon index in patients who did not develop CDI vs. those who did.](image)

0.0019

Patients who did not develop CDI

 Patients who developed CDI


**Patients who develop CDI have significantly lower Shannon index**

![Scatter plot showing Shannon index comparison between patients with and without CDI.](image)

*P <0.005*

*Vincent C, et al. Microbiome. 2013*
Low-Diversity Microbiota & Antibiotic Use are Associated to Colonization to MDROs

Association between low-diversity microbiota (alpha-diversity Shannon index)/antibiotic use and colonization to Multi-Drug Resistant Organisms (MDROs) is well described in the literature:

Carbapenem resistant *Enterobacteriaceae* carriers have significantly lower microbiota diversity compared to other groups

Hospitalized patients colonized with MDROs have significantly lower microbiota diversity compared to hospitalized, non-colonized patients

Prior antibiotic treatment is independently associated with ESBL colonization in ICU patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients colonized to ESBL-PE among prior ABX users</th>
<th>Patients colonized to ESBL-PE among patients who did not use ABX</th>
<th>RR [95% CI]</th>
<th>ABX exposure TW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moustaoui</td>
<td>1/4 (25%)</td>
<td>16/71 (22.5%)</td>
<td>1.1 [0.19-6.39]</td>
<td>NA</td>
</tr>
<tr>
<td>Razazi</td>
<td>16/110 (14.6%)</td>
<td>12/102 (11.8%)</td>
<td>1.24 [0.62-2.49]</td>
<td>Previous year</td>
</tr>
<tr>
<td>Ma.</td>
<td>37/175 (21.1%)</td>
<td>32/287 (11.2%)</td>
<td>1.90 [1.23-2.93]</td>
<td>Last 3 months</td>
</tr>
<tr>
<td>All</td>
<td>1.65 [1.15-2.37]</td>
<td></td>
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</table>

Colonization by Resistant Bacteria is Associated with an Increased Risk of Hospital-Acquired Infections (HAI)

<table>
<thead>
<tr>
<th>ESBL Producing Enterobacteriaceae (ESBL-PE)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled as <strong>Serious Threat</strong> by US CDC</td>
<td></td>
</tr>
<tr>
<td>197,400 cases in-hospital</td>
<td></td>
</tr>
<tr>
<td>9,100 deaths</td>
<td></td>
</tr>
<tr>
<td>$1.2B attributable healthcare costs</td>
<td></td>
</tr>
</tbody>
</table>

Cancer patients with ESBL-PE colonization were 12.98 [95% CI 3.91-43.06] times more likely to develop a BSI with ESBL-PE compared with non-colonized patients.

<table>
<thead>
<tr>
<th>Vancomycin-Resistant Enterococci (VRE)</th>
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<tbody>
<tr>
<td>Labelled as <strong>Serious Threat</strong> by US CDC</td>
<td></td>
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<tr>
<td>54,500 cases in-hospital</td>
<td></td>
</tr>
<tr>
<td>5,400 deaths</td>
<td></td>
</tr>
<tr>
<td>$539M attributable healthcare costs</td>
<td></td>
</tr>
</tbody>
</table>

Dialysis patients with VRE colonization were 21.62 [95% CI 4.33-87.69] times more likely to develop a VRE infection compared with non-colonized patients.

|  |
|------------------|--|
| ICU patients with ESBL-PE colonization were 49.62 [95% CI 20.42-120.58] times more likely to develop an ESBL-PE infection compared with non-colonized patients.

**Sources:**
- Detsis et al., *Crit Care Med.* 2017;
Challenges Met Today Leading to Considering New Endpoints?

- Co-administered prevention approaches reducing dysbiosis and colonization by bacteria and yeasts caused by antibiotics like DAV132 make medical sense.

  ➔ How to envision facilitating their access to market considering microbiological markers as surrogate endpoints to combat urgent threat infections and AMR dissemination?

- Similarly, some Pathogen-Specific Antibacterials in development have shown to have microbiota-sparing properties, expected to lead to reduced risk of selecting for resistance or other colonizing bacterial species, potentially minimizing the overall burden of resistance and subsequent healthcare-associated infections. This represents a very important competitive advantage in classical equivalence phase 3 efficacy study performed for the development of new antibiotics and justifies higher economic valuation on the expected/modelized reduction of secondary infections and hospital dissemination.

  ➔ How to include new achievable biomarkers, such as colonization with bacterial species associated with morbidity and mortality risks (MRSA, VRE, C. diff, etc.), which could then be described within the clinical section of the label?

- Finally, decolonization strategies make medical sense too.

  ➔ Would microbiological carriage endpoints could be considered for clinical development of HAI prevention products via decolonization MoA? Alignment with EMA guideline*?

*EMA Guideline on the evaluation of medicinal products indicated for treatment of bacterial infections (CPMP/EWP/558/95 Rev 3)
Take-Home Messages

• Sparing the microbiota from antibiotic-associated dysbiosis and colonisation by resistant micro-organisms is possible technically and pharmacologically, however not yet financially.

• Current regulations do not allow for feasible clinical developments because demonstrating a reduction of colonisation followed by a reduction of secondary infections and dissemination necessitates too large and expensive studies.

• New regulations accepting prevention of colonisation as an endpoint for clinical development are a necessity to make available such a strategy for the benefit of individual patients and the global control of AMR.
About DAV132


• de Gunzburg et al. *Protection of the human gut microbiome from antibiotics.* The Journal of Infectious Diseases. 2018. Link


• de Gunzburg et al. *Targeted Adsorption of Molecules in the Colon with the Novel Adsorbent-Based Medicinal Product, DAV132: A Proof of Concept Study in Healthy Subjects.* J Clin Pharmacol. 2015. Link

• Saint-Lu N et al. *DAV131A protects hamsters from lethal Clostridioides difficile infection induced by fluoroquinolones.* Antimicrobial Agents and Chemotherapy. 2019. Link

• Grall et al. *Oral DAV131, a Charcoal-based Adsorbent, Inhibits Intestinal Colonization by Beta-lactam Resistant Klebsiella pneumoniae in Cefotaxime-Treated Mice.* AAC. 2013. Link

About Immuno-Oncology


About Hemato-Oncology

• Lurienne L, et al. *Incidence of CDI in a cohort of US patients with newly diagnosed Acute Myeloid Leukemia receiving intensive chemotherapy.* ASH 2020, poster 3406. Link


• Duhalde et al. *Excess burden associated with Clostridioides difficile infection in haematological patients occurring during hospitalization with induction chemotherapy in the United States.* J Hosp Infect. 2019. Link