# ALTERNATIVE PRESERVATIVES FOR VACCINES

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# ....CONCLUSIONS.....

There is currently no...

- Ideal (alternative) antimicrobial preservative (AP) for vaccines
- AP gold standard for vaccines
- 'R&D pipeline' for vaccine AP

Lack of selective affinity of AP (≠ anti-infectives)

tough AET criteria & other RA requirements

vaccines are tough substrates for AP:
- narrow range for pH, *I*, °T
- interference due to excipients &
protein immunogen (vaccine cornerstone!)

*Product quality & potency = ?*

Allergy/ Hypersensitivity?
(immunological promiscuization and bystander Th)

# (RA) REQUIREMENTS TO VACCINE PRESERVATIVES

- **Stable** and effective in the formulated vaccine throughout its shelf life at the labeled storage conditions. (2-8 °C)
- **Quality** appropriate as demonstrated by characterization data & compliance with specs (including side products, purity profile, protein integrity)
- Compliance with **AET criteria** (requires *broad antimicrobial* spectrum and – in case of ESP (criteria A) and WHO recommendations re: OVP: *microbicidal activity* + *fast onset)*
- **safe** as shown by in pre-clinical toxicology studies.
- **No interference** with vaccine stability or immunogenicity (*in vitro* antigenicity, potency and preclinical immunogenicity as compared to preservative-free vaccine or licensed preservative-containing vaccine)
- Available at **low cost**
- Compliance with RA guidance may require ***clinical studies***: case-by-case

→ testing & screening to be started as of early phases of product dvpt!

# IMPACT OF PRESERVATIVES ON PROTEIN INTEGRITY

Effect of Preservatives on Protein Stability and Antimicrobial Efficacy*

| Preservative | Concentration(%) | Monomer (%), 45ºC | Potency (%), 45ºC | Bacteria x $10^4$ (cfu/mL), † 25ºC | Fungi x $10^5$ (cfu/mL), ‡ 25ºC |
|---|---|---|---|---|---|
| Benzyl alcohol | 0.75 | 87.9 | 74 | 0 | 0 |
| | 0.5 | 93.9 | 75 | 0 | 0 |
| | 0.1 | 97.7 | 81 | TNTC | 12 |
| Chlorobutanol | 0.2 | 96.7 | 63 | 46 | 0 |
| | 0.1 | 97.5 | 67 | TNTC | 14 |
| | 0.05 | 97.6 | 60 | TNTC | 18 |
| Methylparaben | 0.1 | 97.2 | 75 | 56 | 0 |
| | 0.05 | 97.4 | 75 | TNTC | 2 |
| | 0.01 | 98.3 | 73 | TNTC | 26 |
| Propylparaben | 0.01 | 97.3 | 68 | TNTC | 0 |
| | 0.0075 | 97.9 | 74 | TNTC | 2 |
| Control | — | 97.5 | 69 | TNTC | 20 |

Positive correlation between concentration of AP and degree of protein alteration

(S. Gupta, AAPS PharmSci 5; 2003)

# IMPROVING ANTIMICROBIAL EFFECTIVENESS: A REALISTIC GOAL?

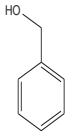
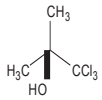
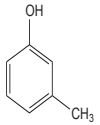
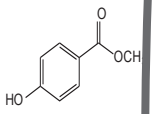
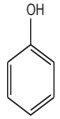
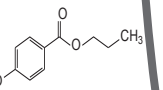
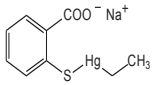
Use other, conventional APs? ☹ ....since all featured by lack of selective affinity .....

How to enhance selective affinity?

New approaches to improving antimicrobial effectiveness:

- Change phase behavior via combinations of APs (e.g., CB/ BA + parabens)
- Via use of <u>new, other compounds</u> (e.g., cationic antimicrobial peptides, CAPs)
- *Rely on characteristics of <u>active ingredients, e.g., Ag itself</u> (e.g., PS, DNA,...) or Ag carrier*:

  (i.e., prevent interaction of AP with Ag ± increase local concentration & bioavailability of AP at level of MO)
-

| Preservative Name and Typical In-Use Concentration[5] | Chemical Structure[7] | Antimicrobial Activity (Minimum Inhibitory Concentration (MIC), mg/mL)[3] Gram Positive Bacteria (S. aureus) | Gram Negative Bacteria (E. coli/P. aeruginosa) | Yeast (C. albicans) | Mold (A. niger) | Optimal pH[3] |
|---|---|---|---|---|---|---|
| Benzyl alcohol 1% |  | 25 | 2000/2000 | 2500 | 5000 | <5 |
| Chlorobutanol 0.3–0.5% |  | 650[a] | 1000[a] | 2500[a] | 5000[a] | <5.5 |
| m-cresol 0.3% |  | Not specified | Not specified | Not specified | Not specified | <9 |
| Methylparaben 0.2% |  | 2000 | 1000/4000 | 2000 | 1000 | 4–8 |
| Phenol 0.25–5% |  | Not specified | Not specified | Not specified | Not specified | <9 |
| Phenoxyethanol 1% |  | 8500 | 3600/3200 | 5400 | 3300 | <7 |
| Propylparaben 0.2% |  | 500 | (100–500)/(>1000) | 250 | 200–500 | 4–8 |
| Thimerosal 0.002–0.01% |  | 0.2 | 4/8 | 32 | 128 | 7–8 |

[a]MIC values are not specific for S. aureus, E. coli, P. aeruginosa, C. albicans, or A. Niger.

Chemical structures and antimicrobial activity of preservatives commonly used in parenteral biological products

(Meyer et al. J.Pharm. Sc. 96 ;2007)

# THE 'PIPELINE' OF VACCINE PRESERVATIVES?

Use conventional preservatives from licensed non-vaccine parenteral formulations?

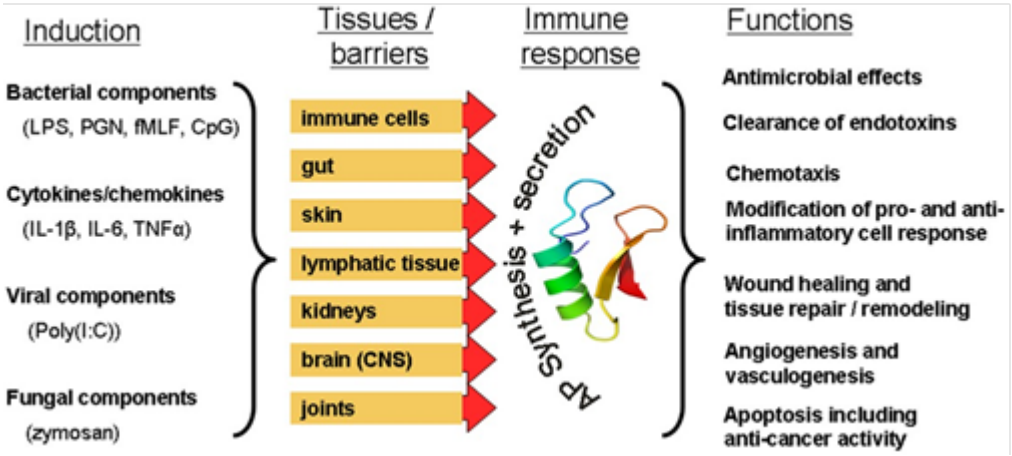
| Preservative name and typical in-use concentration (w/v) | Use in peptide-/ protein-containing parenteral formulations | Use in vaccines |
|---|---|---|
| Benzyl alcohol 1% | yes | no |
| Chlorobutanol 0.3-0.5% | no (1 exception) | no |
| Phenol 0.25-0.5% | yes | yes |
| m-Cresol 0.1-0.3% | yes | no |
| Methylparaben 0.1-0.2% | no | no |
| Propylparaben 0.01-0.02% | no | no |
| 2-Phenoxy-1-ethanol 0.5-1% | yes | yes |
| Benzethonium chloride 0.01-0.02% | no | no (1 exception) |

List of alternative preservatives commonly used in parenteral formulations.
Only some of them (marked with 'yes') are regularly used in biologicals

# THE LEXICON OF VACCINE PRESERVATIVE TESTING

- Cost-intensive and
- Time-consuming and
- Empirical (trial and error) and
- Unpredictable and
- Case-by-case and…
- Not considered a priority….

# HOW TO ENHANCE SELECTIVE AFFINITY?
# USE NEW COMPOUNDS: CATIONIC ANTIMICROBIAL PEPTIDES?




Brandenburg et al., Polymers 4 (2012)

Induction, examples of localization and potential biological roles of cationic antimicrobial peptides

# CAPS: PRESERVATIVE-RELEVANT CHARACTERISTICS

- **Different classes:**

  'natural' cathelicidins, defensins, histatins

- **Physicochemical characteristics :**

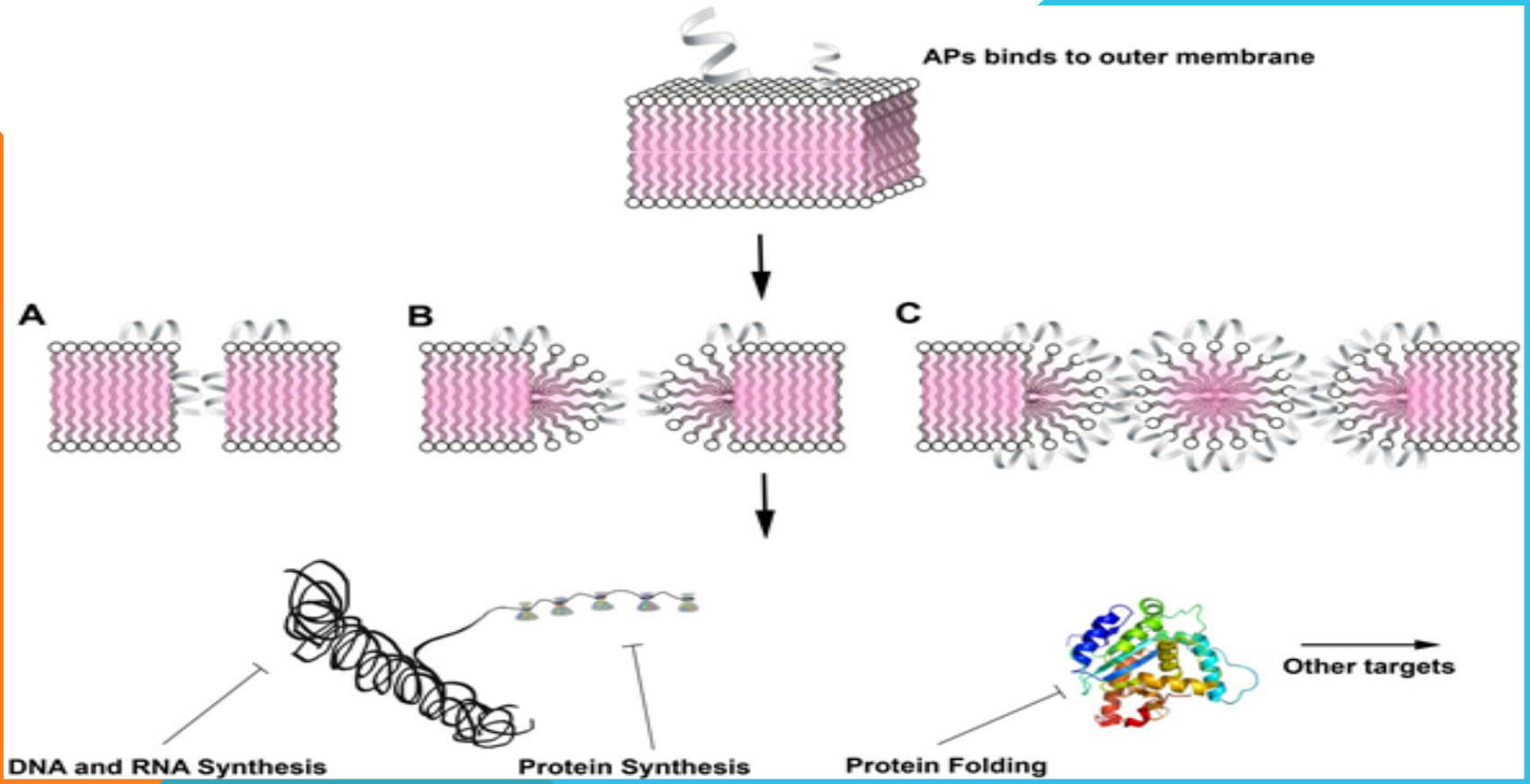
  - short (10-50 amino acids; MW: 10kDa)
  - high proportion of + charges (arginine/ lysine)
  - high proportion of hydrophobic moieties

  ⇩ 'unusual' amphiphilic properties

Higher selective affinity to conserved & accessible microbial determinants

**PROS**: more discriminative, broad-spectrum, fast, onsett, low risk of antimicrobial resistance

# CAPS: MECHANISM OF ACTION




Perturbation of extracellular and intratracellular structures (e.g., membrane destabilization/ permeabilization and interaction with DNA synthesis &protein folding)

Brandenburg et al., Polymers 4 (2012)

# CAPS: HOW DO THEY DIFFER FROM ANTIBIOTICS?

Mechanism of action and resistance of antibiotics (ABs) as compared to CAPs

| | |
|---|---|
| Inhibition of cell wall synthesis: | applies to ABs |
| Inhibition of protein synthesis (translation): | applies to ABs |
| Alteration of cell membranes: | applies to Abs & CAPs (e.g., polymyxin is listed as AB!) |
| Inhibition of nucleic acid synthesis: | applies to Abs & CAPs |
| *Anti-metabolite activity:* | applies to Abs & ?? *CAPs* |

# CAPS: PERFORMANCE IN AET

| Time | Compounds | Number of microorganisms per gram of test solution | | | | |
|---|---|---|---|---|---|---|
| | | *E. coli* ATCC 8739 | *S. aureus* ATCC 6538 | *P. aeruginosa* ATCC 9027 | *C. albicans* ATCC 10231 | *A. niger* ATCC 16404 |
| 0 h | | $2 \times 10_5$ | $10_5$ | $2{,}6 \times 10_5$ | $3{,}1 \times 10_5$ | $1{,}4 \times 10_5$ |
| 6 h | Citropin | $4{,}2 \times 10_5$<br>$4{,}6 \times 10_5$ | $2 \times 10_4$/- | $10_4$<br>$5 \times 10_4$ | -/- | -/- |
| | Protegrin | $1{,}9 \times 10_5$<br>$2{,}4 \times 10_5$ | $10_4$/- | $1{,}6 \times 10_5$<br>$2{,}1 \times 10_5$ | -/- | -/- |
| | Citropin + Protegrin | $2{,}6 \times 10_5$<br>$3{,}2 \times 10_5$ | -/- | $9 \times 10_4$<br>$1{,}6 \times 10_5$ | -/- | -/- |
| | Benzalkonium chloride | -/- | -/- | -/- | -/- | -/- |
| 24 h | Citropin | $5 \times 10_4$/- | -/- | -/- | -/- | -/- |
| | Protegrin | $2 \times 10_4$<br>$3 \times 10_4$ | -/- | -/- | -/- | -/- |
| | Citropin + Protegrin | $2 \times 10_4$<br>$5 \times 10_4$ | -/- | -/- | -/- | -/- |
| | Benzalkonium chloride | -/- | -/- | -/- | -/- | -/- |
| 2 days | Citropin | $10_4$/- | -/- | -/- | -/- | |
| | Protegrin | -/- | -/- | -/- | -/- | -/- |
| | Citropin + Protegrin | -/- | -/- | -/- | -/- | -/- |
| | Benzalkonium chloride | -/- | -/- | -/- | -/- | -/- |

AET on CAPs (citropin, protegrin separate or in mixture) in sterile water
(Acta Pol. Pharmac. 62; 2005).

# CAPS: ANTIMICROBIAL ACTIVITY

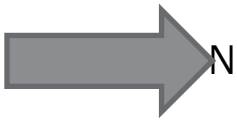
| Compound | Minimal inhibitory concentration and minimal bactericidal or fungicidal concentration (MIC/MBC) [µg/mL] | | | | |
|---|---|---|---|---|---|
| | *E. coli* ATCC 8739 | *S. aureus* ATCC 6538 | *P. aeruginosa* ATCC 9027 | *C. albicans* ATCC 10231 | *A. niger* ATCC 16404 |
| Citropin 1.1 | 128/256 | 8/8 | 256/256 | 16/16 | 32/32 |
| Protegrin 1 | 4/4 | 8/8 | 8/16 | 8/8 | 64/128 |
| Benzalkonium chloride | 16/16 | 2/2 | 32/32 | 8/8 | 64/64 |

Antimicrobial activity of CAPs against reference strains
(Acta Pol. Pharmac. 62; 2005).

# CAPS: OUTSTANDING QUESTIONS

## RE: SAFETY, EFFICACY & TECHNICAL FEASIBILITY

- Antimicrobial effectiveness requires high concentration (in mg/ ml!)
- Salts /ions and lipids may negatively impact on activity
- Stability (degradation) may be issue unless synthetic bio-/peptidomimetics
- ? Cost (screening, synthesis)
- ? Safety (may trigger innate immune signaling cascades)
- (? Resistance)



NOT ready to go……

# IMPROVE ANTIMICROBIAL EFFECTIVENESS (AE) VIA ACTIVE INGREDIENTS….
# 2-PE VS THIMEROSAL: AE IN CONJUGATE POLYSACCHARIDE VACCINE

(KHANDKE ET AL., VACCINE 29; 2011)

Preservative effectiveness in various formulations.

| Formulation | Thimerosal Hg con. 1-g/dose (%) Microgr./dose (%) | Measured Hg con. 1- | 2-PE mg/dose | | Multi-challenge | Single challenge | Multi-challenge | Single challenge |
|---|---|---|---|---|---|---|---|---|
| | | | | | EP (A) | EP (A) | EP (B) | EP (B) |
| Prev(e)nar 13™ | 0 | 0 | 5 | 4.8 | Pass | Fail | Pass | Pass |
| Prev(e)nar 13™ | 0 | 0 | 7.5 | 7.3 | Pass | Pass | Pass | Pass |
| Prev(e)nar 13™ | 100 (0.04) | ND | 0 | 0 | ND | Fail | ND | Pass |
| Prev(e)nar 13™ | 501-g (0.02) | 41.4 ± 2.9 | 0 | 0 | Fail | Fail | Fail | Pass |
| Prev(e)nar 13™ | 251-g (0.01) | 22.0 ± 3.6 | 0 | 0 | Fail | Fail | Fail | Fail |
| Saline control | 501-g (0.02) | 43.2 ± 3.7 | 0 | 0 | Fail | Fail | Fail | Pass |