CLSI Symposium

Biofilm susceptibility testing: Relevance and concerns for product development
21st January 2012

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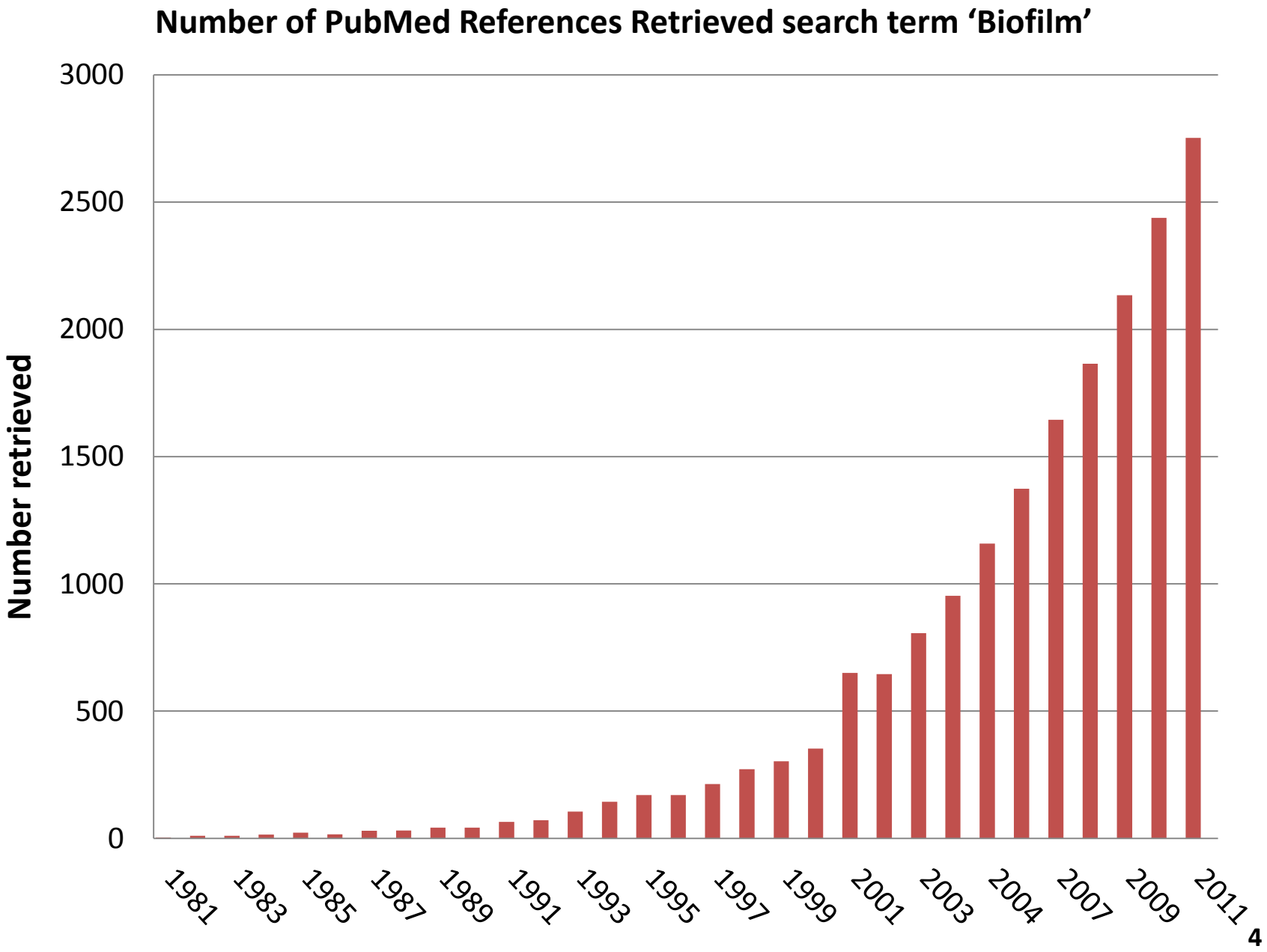
Disclosures

I am the Chief Scientific Officer at Euprotec Ltd that provides fee-for service research for a wide range of Biotech and Pharmaceutical companies under confidentiality agreements.

The views expressed in this presentation are my own.

Overview

- Are biofilms important
- What do the regulators want
- What does industry want
- How can industry fulfil the regulators requirements


Number of PubMed References Retrieved search term 'Biofilm'
Number retrieved
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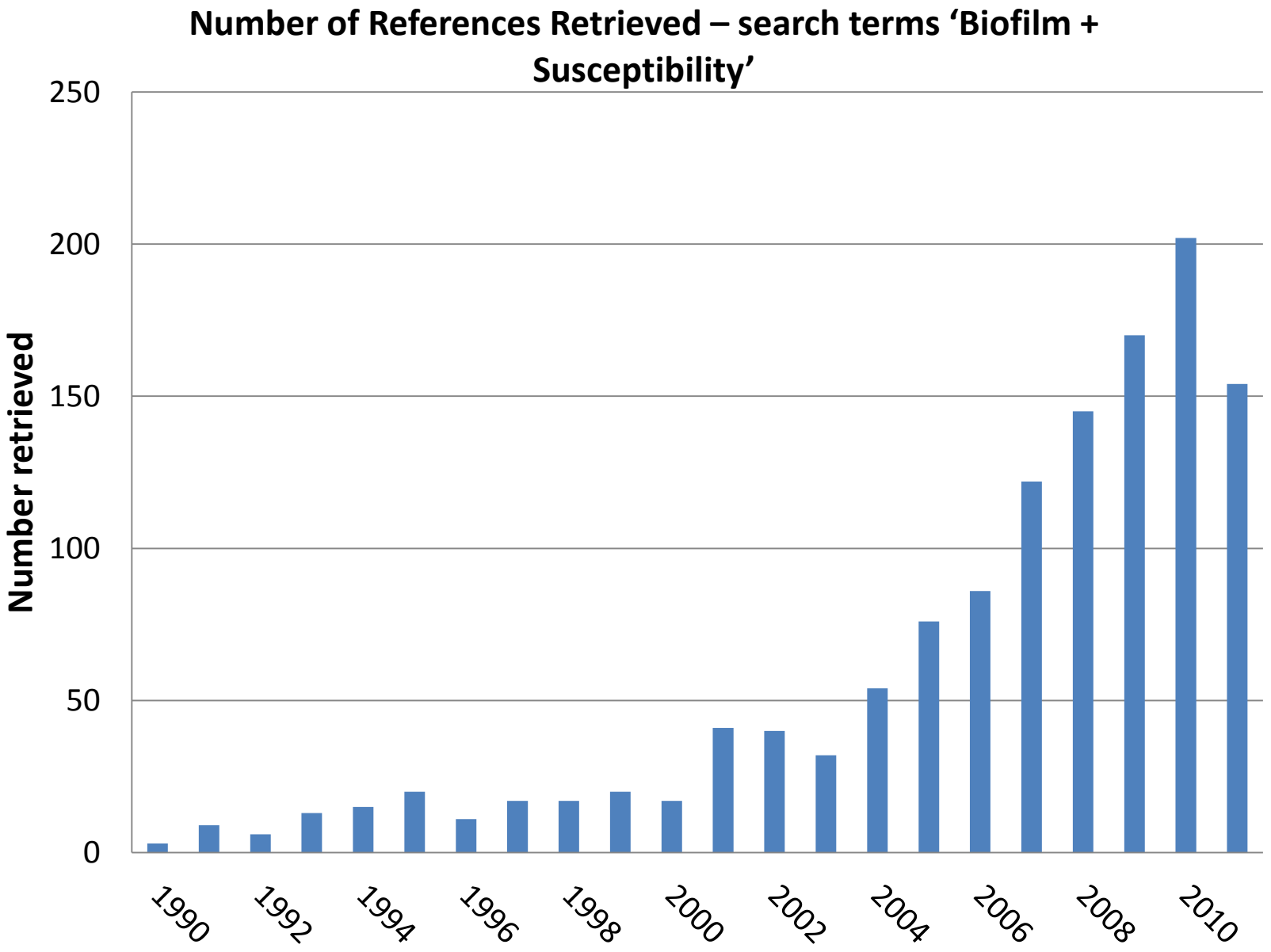
Number of References Retrieved – search terms 'Biofilm + Susceptibility'
Number retrieved
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200
150
100
50
0
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
2010

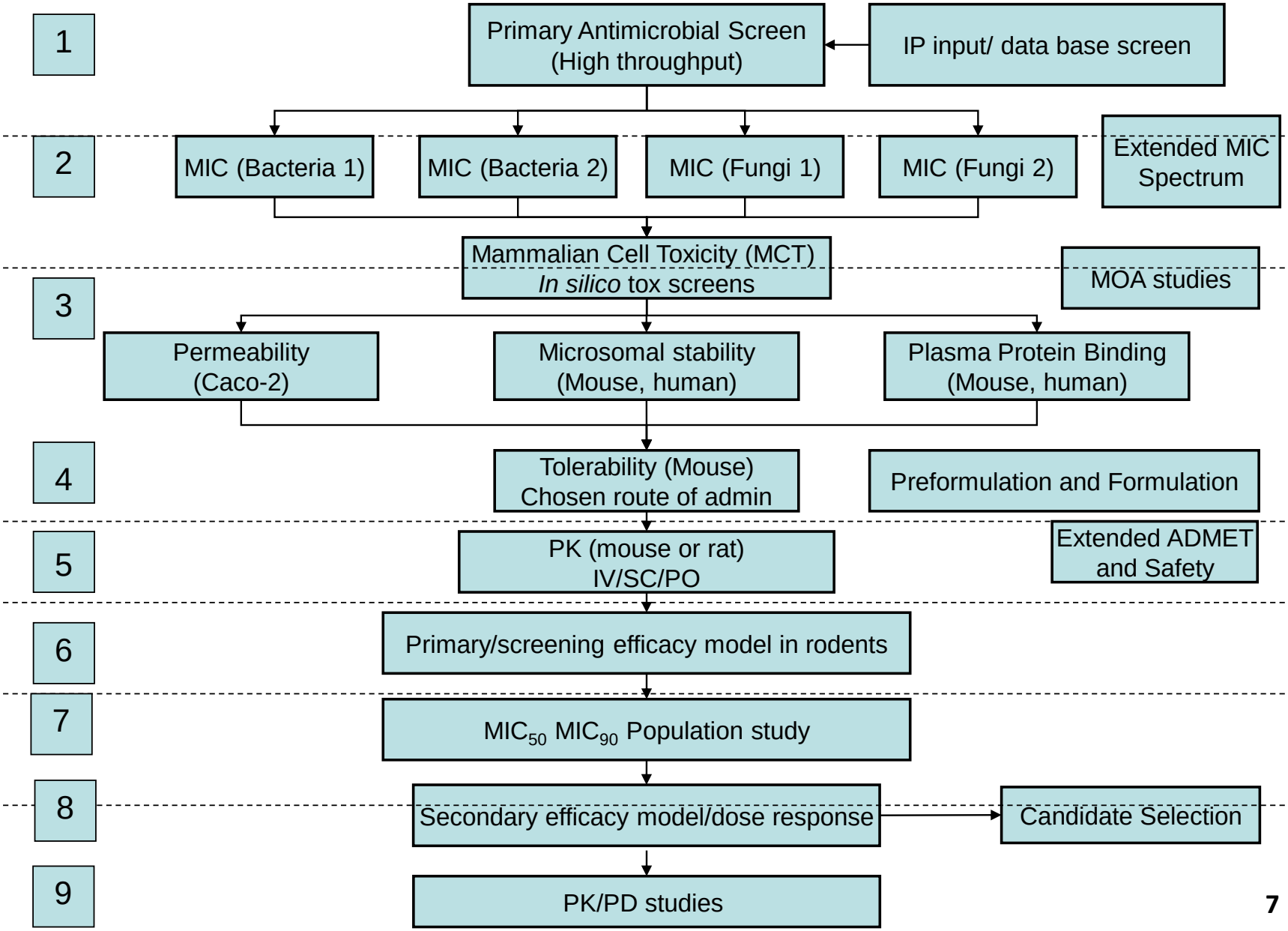
Alternative PubMed Searches

- Biofilm device 4419
- Candida biofilm 942
- Bacterial biofilm 10216
- Staphylococcal biofilm 2013

Most agree that biofilms are commonly associated with infections, are difficult to treat and cause significant morbidity and mortality.

Therefore as biofilms are important we need drugs to treat them.

Hypothetical Drug Screening cascade (incomplete) to develop a candidate drug



Where are Guidelines needed in a screening/drug development cascade? What does industry want

Hit finding/ high throughput screening – method does not need a guideline as it is an indication of activity only, typically screened in 96/384 well plates. *But a method would be helpful*

Primary screen/ preliminary profile - *A* standard method would be helpful but not mandatory

Secondary Screen/ extended range
Standardized methods essential

MIC50/MIC90/Spectrum of activity-Standardized methods essential

MOA, mechanism of resistance, PK/PD- Standardized methods essential

REGULATORY GUIDANCE

FDA Guidance for Industry
Microbiological Data for Systemic Antibacterial Drug Products — Development, Analysis, and Presentation

DRAFT GUIDANCE September 2009

EMEA Guideline on the evaluation of medicinal products indicated for treatment of bacterial infections

Effective from 15 January 2012

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003417.pdf

REGULATORY GUIDANCE

FDA Sponsors should describe the methods used for generating susceptibility data. If a recognized reference method is used, sponsors can **reference the standard method.** However, if susceptibility data are obtained by modification of a standard method, or by other methods, sponsors should provide a *detailed description of the method, including the justification for the modification of the method, the effect on susceptibility results, and validation of the method.*

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf

REGULATORY GUIDANCE

EMEA During the microbiological and clinical development programmes the sponsor should collect sufficient data to characterise the *in-vitro* antibacterial activity against recent clinical isolates. The preferred method and extent of susceptibility testing should be in accordance with the recommendations of EUCAST.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003417.pdf

REGULATORY GUIDANCE

Compliance with both sets of regulators is difficult as they require the generation of different data sets entailing additional costs and delays.

BUT there are some get-out-of-jail clauses.
As the science of clinical microbiology and the development of antibacterial drug products evolve, ***this guidance will be revised.*** *We recognize that the* ***results of in vitro susceptibility testing are not absolute*** *for a variety of clinical and technical reasons and are meant only to guide treatment. The accuracy and clinical relevance of such tests depend* ***on adherence to standardized methods*** *and appropriate consideration of the test results*.

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REGULATORY GUIDANCE

Microbiology package FDA:

In vitro microbiological data and *in vivo* animal studies (e.g., **spectrum of activity *in vitro*** and appropriate animal models of human disease) support the justification of testing in humans.

.....Microbiological data submitted to an NDA will be used to substantiate the microbiological information **contained in the labeling for human prescription drugs** and biological products (labeling).

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This means that is you want to claim activity against biofilms on the label you need to prove it!

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf

REGULATORY GUIDANCE

Microbiology package FDA:

….discusses the following specific microbiological issues that should be addressed in the NDA:

- Spectrum of antimicrobial activity. *How do we know results are reliable if there is no reference method?*
- Other anti-infective properties (e.g., MOA, mechanism of resistance, activity in the presence of body fluids, development of hetero-resistance) *Active against biofilms?*
- Methods for *in vitro* susceptibility testing
- Proposed quality control (QC) for susceptibility testing. *Which isolates?*
- Proposed interpretive criteria for susceptibility test results
- ….appropriate animal models of infection that support proof of concept
- Information from clinical trials evaluating clinical outcome by baseline **pathogen MIC data**

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf

REGULATORY GUIDANCE

Microbiology package FDA:

….discusses the following specific microbiological issues that should be addressed in the NDA:

- **Specify the method** by which *in vitro* activity of the antibacterial drug product can best be determined (e.g., microbroth dilution, disk diffusion)
- Evaluate **culture and environmental conditions** that may affect the assessment of *in vitro* antibacterial activity
- Establish **QC parameters** for *in vitro* susceptibility testing of the antibacterial drug product before determining its activity against bacterial isolates
- Demonstrate *in vitro* activity against target bacteria
- Determine equivalence between broth dilution and agar dilution susceptibility test results

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REGULATORY GUIDANCE

Microbiology package FDA:

….discusses the following specific microbiological issues that should be addressed in the NDA:

- *In vitro* activity of the antibacterial drug product in the presence of human body fluids and secretions.
- Determine if interactions with other antimicrobial agents (e.g., antagonism, synergy, additive) and nonantimicrobial drugs (e.g., interference) might occur
- Provide information on mechanisms of action and on the potential for the development of resistance and cross-resistance to other antimicrobials

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ALL OF THIS WILL NOT BE POSSIBLE IF APPROPRIATE GUIDELINES ARE NOT IN PLACE

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REGULATORY GUIDANCE

Microbiology package FDA:

For the following reasons this might be tough

✓Tested with a comparator against at least 500 isolates

Method selected must be suitable for rapid throughput

✓Sufficient range of clinically relevant bacteria *What should the range be for biofilm isolates?*

✓Include the prominent genotypes, serotypes, biotypes, and isolates with known mechanisms of resistance.

The MIC range and the number of isolates tested

MIC50

MIC90

MIC:MBC ratio for members of clinically relevant genera

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf

REGULATORY GUIDANCE

Microbiology package FDA:

QC Procedures

QC parameters ……..to ensure the generation of precise, accurate, and reproducible results.

If susceptibility information provided is obtained without proper quality **monitoring…results may be considered invalid**.

Routine QC …..testing of designated well characterized QC strains

10 replicates of each QC strain over 3 days in at least 7 different laboratories

If a QC microorganism is chosen that is different from an existing FDA-recommended QC microorganism, it should be **deposited in a recognized culture collection**

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REGULATORY GUIDANCE

Microbiology package FDA:

Animal Therapeutic and Pharmacological Studies

To correlate *in vitro* and in vivo activity , sponsors should consider the use of appropriate animal models of infection for systemic antibacterial drug products when studying the PK/PD and activity of antibacterial drug products.

Determination of the pharmacodynamic driver requires a precise MIC

e.g. AUC/MIC or T>MIC parameters plus magnitude studies

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM182288.pdf

REGULATORY GUIDANCE

EMEA Guideline

Similar to the FDA guidelines there is no specific mention of biofilms

But there is a greater specific emphasis on PK/PD studies particularly when clinical trial would be difficult
Based on **in-vitro susceptibility** test data, information from non-clinical models of efficacy and human PK data, detailed PK/PD analyses may be used to support dose regimen selection and susceptibility testing breakpoints.
In circumstances in which it is not feasible to generate extensive clinical efficacy data ……..PK/PD analyses may also provide important **supportive information on the likely efficacy of the test antibacterial agent.**

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003417.pdf

REGULATORY GUIDANCE

EMEA Guideline

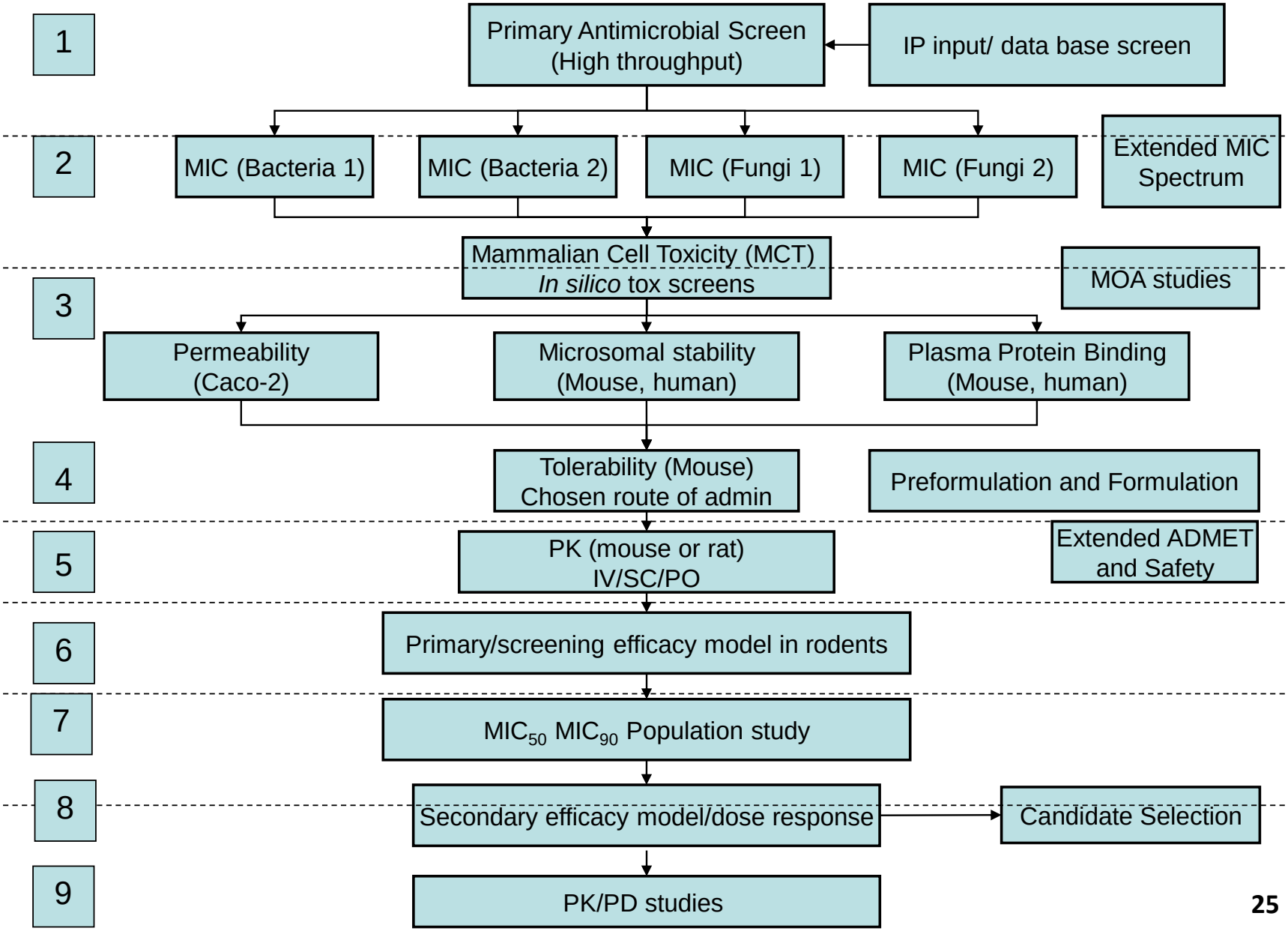
PK/PD (cont)

The overall assessment of the PK/PD should be sufficiently comprehensive to assess ….whether or not the test antibacterial agent…..would have useful clinical activity against relevant pathogens that appear to be **susceptible *in vitro***.

The **MIC distributions** for wild-type populations of pathogens relevant to the **indications sought** should be taken into account so that the PK/PD analyses cover the highest MICs considered to be treatable with well-tolerated dose regimens. *To my knowledge there have been no such studies performed so new methods need to be agreed.*

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003417.pdf

Hypothetical Drug Screening cascade (incomplete) to develop a candidate drug



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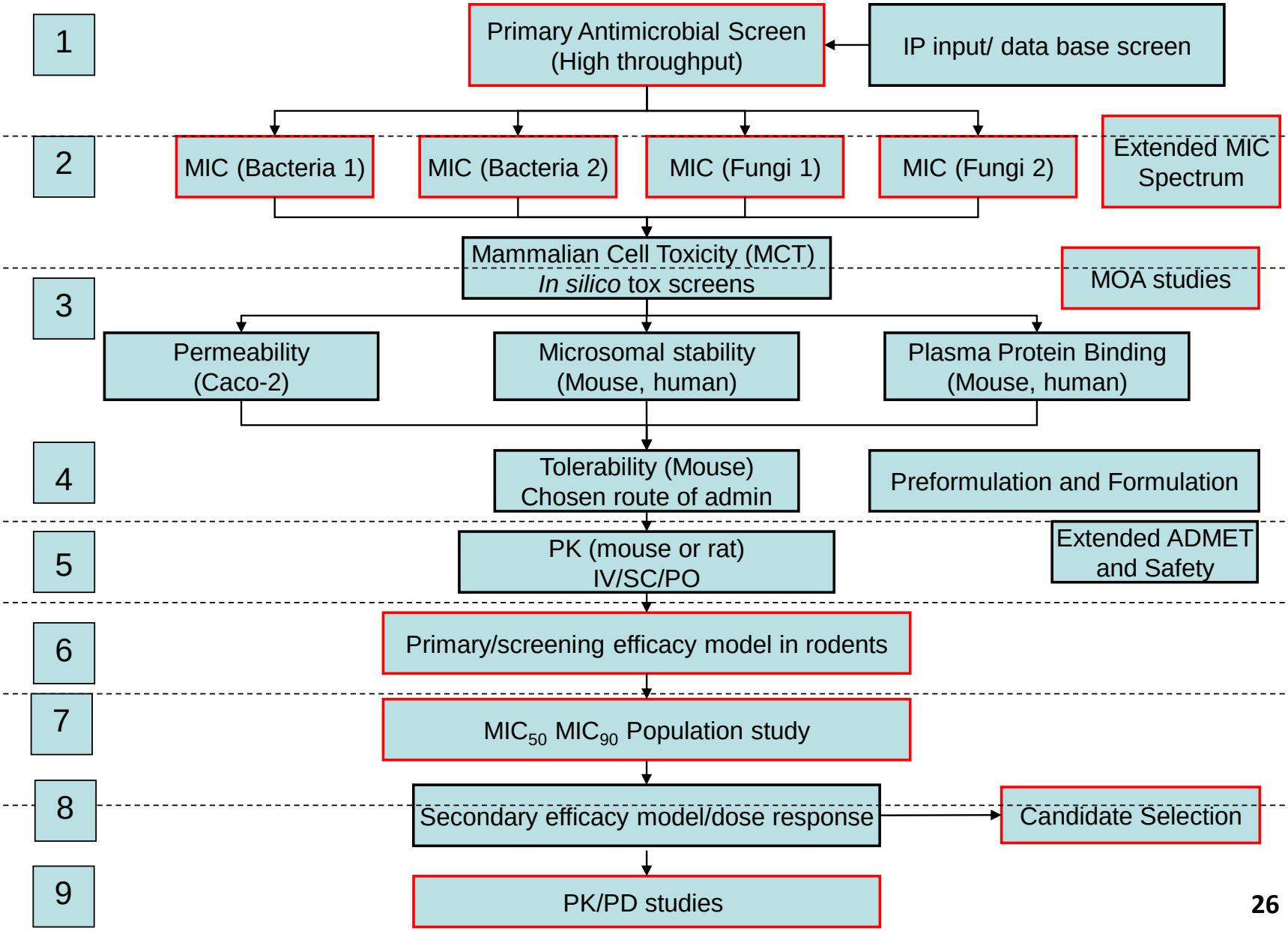

Stages

- Stage 1
  - Primary antimicrobial Screen
    - Against bacteria or fungi
    - Single concentration
    - Hit finding High throughput

Method need to be suitable for high-throughput but sufficiently predictive for general use

- Stage 2
  - Secondary screen (confirmation)
  - Minimum Inhibitory Concentration (MIC)
  - Selected pathogens according to requirements
  - Extended spectrum profiling where appropriate (broad panel)

If possible should be aligned with standard guidelines

- Stage 3
  - eADME
  - Cytotoxicity counterscreening (selectivity)
  - Metabolic stability (*in vitro*)
  - Plasma protein binding
  - CaCo-2 (if appropriate)

Stages

- Stage 4
  - Tolerability (*in vivo*)
  - Single or repeat dose
  - Select compounds for PK – which administration routes are suitable
  - Preliminary Pre-formulation and formulation
    - Solubility/lipophilicity/pka screening where appropriate

- Stage 5
  - Single dose PK, single species
  - Ensure exposure *via* chosen route for efficacy studies
  - Extended ADMET and Safety

- Stage 6
  - Efficacy (primary, screening model)
    - Ascertain effect in infection model
    - Higher throughput
    - Select compounds for more detailed efficacy experiments

Stages

- Stage 7

  Detailed *in vitro* susceptibility/population studies MIC_{50}/MIC_{90}.

  Generation of resistant mutants

  Essential this should be aligned with standard guidelines

- Stage 8
  - Secondary Efficacy Model
    - Confirm activity
    - Establish dose response relationship
  - Detailed PK profiling
    - Second species
    - Repeat dose
    - Ascending dose
    - Tissue distribution

Stages

- Stage 8 (continued)
  - Candidate selection activities
    - Extended ADMET and Safety profiling
      - Drug interaction studies
        - » CYP inhibition, induction, and isoform mapping
        - » P-gp profiling
      - Preliminary genotox (screening Ames)
      - Cytotoxicity screening against human cell lines
      - hERG (patch-clamp)
    - Predevelopment Tolerability
      - 7-14d Rat tolerability study
  - Pre-formulation and formulation
    - Detailed physicochemical properties
- Stage 9
    - PK/PD studies, magnitude of effect
    - Defined susceptibility testing methods

Essential this should be aligned with standard guidelines

REGULATORY GUIDANCE

FDA Guidance for Industry

Catheter-Related Bloodstream Infections — Developing Antimicrobial Drugs for Treatment

DRAFT GUIDANCE October 1999

Only one mention of Biofilm and no reference to susceptibility testing

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070972.pdf

Where are Guidelines needed in a screening/drug development cascade? What does industry want

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ALL OF THIS WILL NOT BE POSSIBLE IF APPROPRIATE GUIDELINES ARE NOT IN PLACE Therefore, in answer to the question is a new CLSI Guideline required - the answer is a clear YES

essential

MOA, mechanism of resistance, PK/PD- Standardized methods essential

Acknowledgements

Thanks to the team at Euprotec particularly Dr Lloyd Payne for the permission to share his slides on drug screening cascades.