

## *Pseudomonas aeruginosa*

combattre la virulence : de nouvelles voies thérapeutiques ?

Eric Kipnis

EA 7366 - Recherche translationnelle : relations hôte-pathogènes  
Faculté de Médecine de Lille UDSL - Univ Lille Nord de France

# Conflits d'intérêts

---

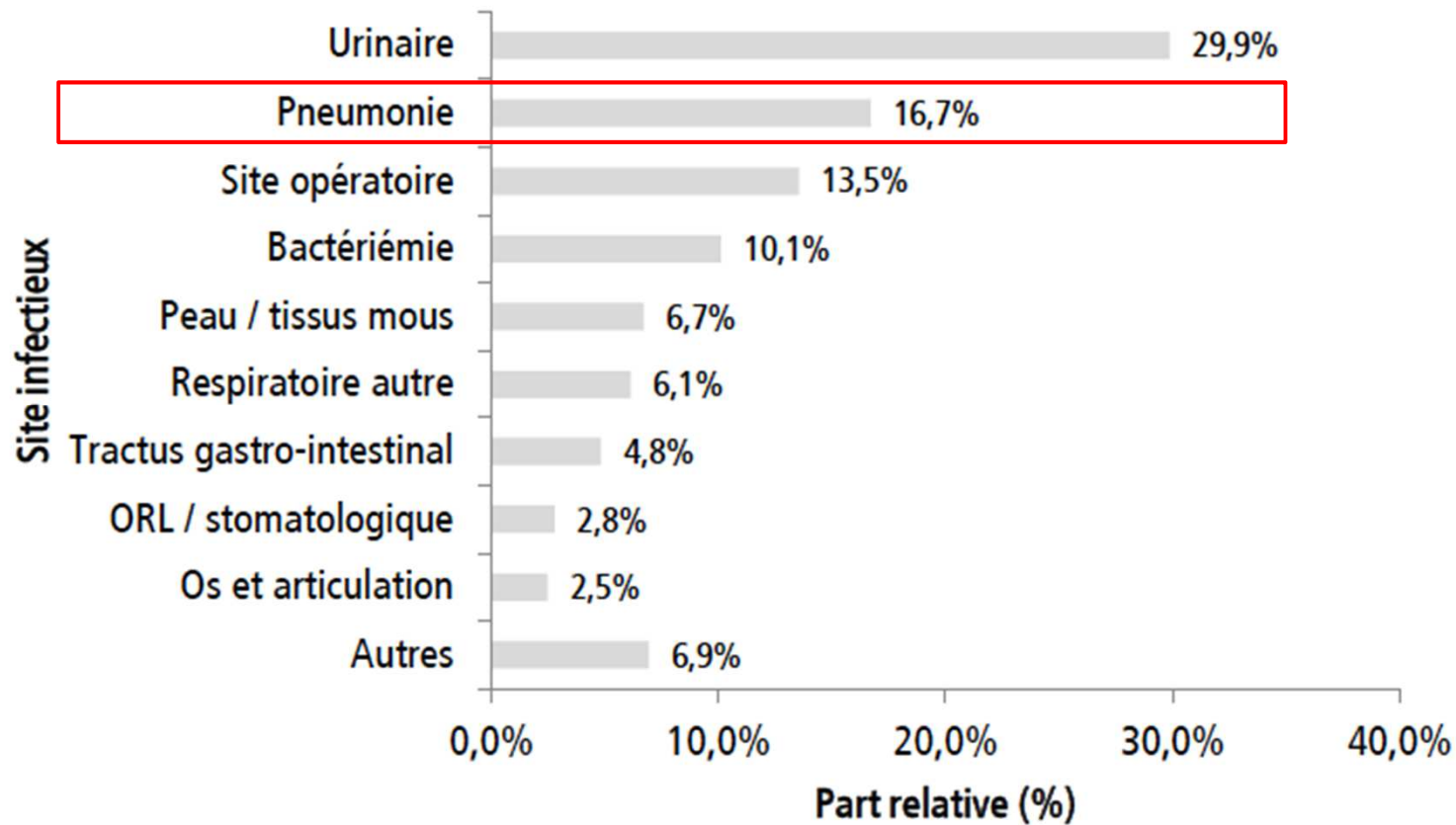


- Comités de pilotage : Fresenius
- Investigateur : KaloBios, Biomérieux, Méditor, Fresenius
- Orateur : LFB, Gilead, MSD
- Meetings et congrès : Pfizer, MSD, Gilead, Astra-Zeneca, LFB, Braun

# Problématique

## Epidémiologie : pneumopathies nosocomiales

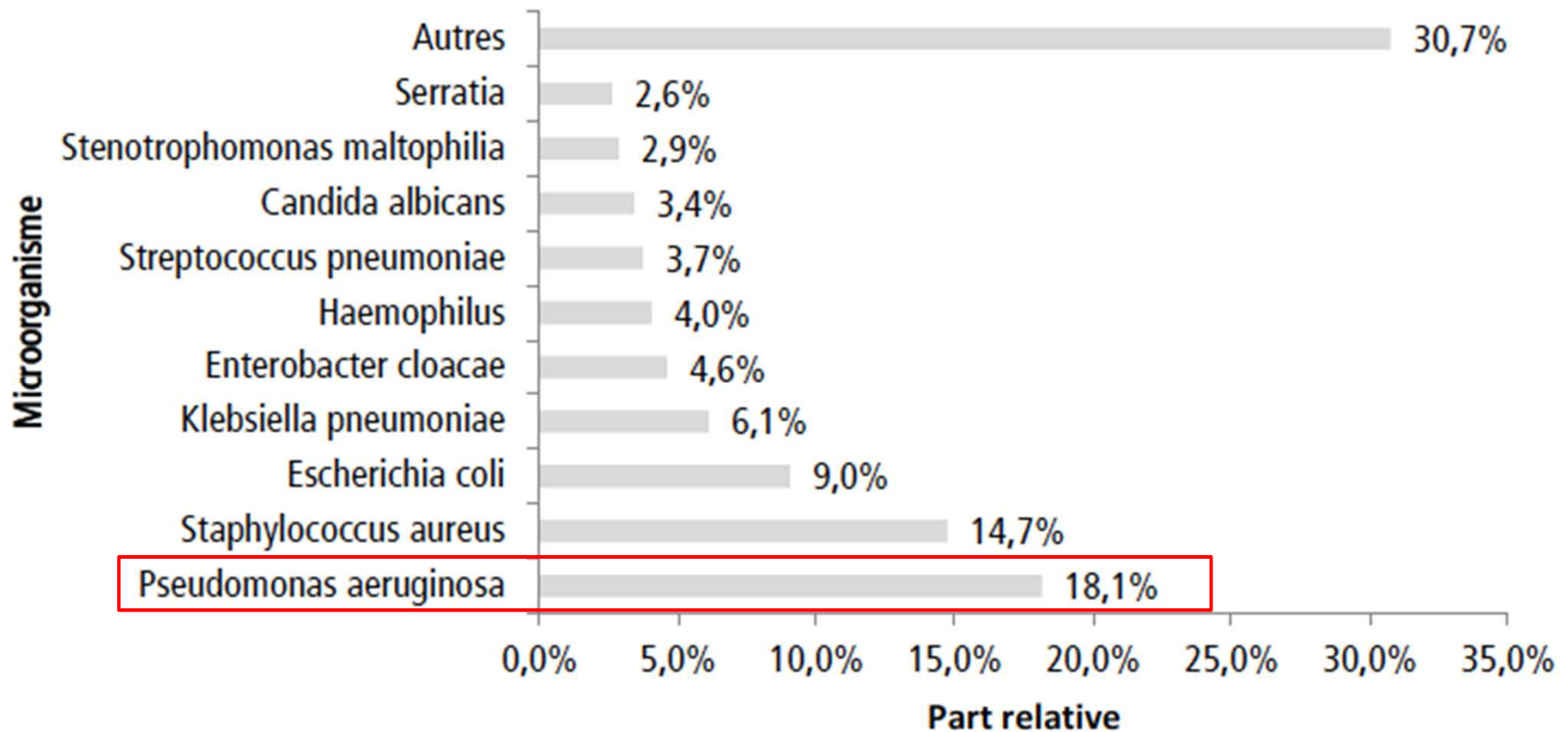
enquête nationale de prévalence 2012



# Problématique

## Epidémiologie : pneumopathies nosocomiales

enquête nationale de prévalence 2012



# Problématique

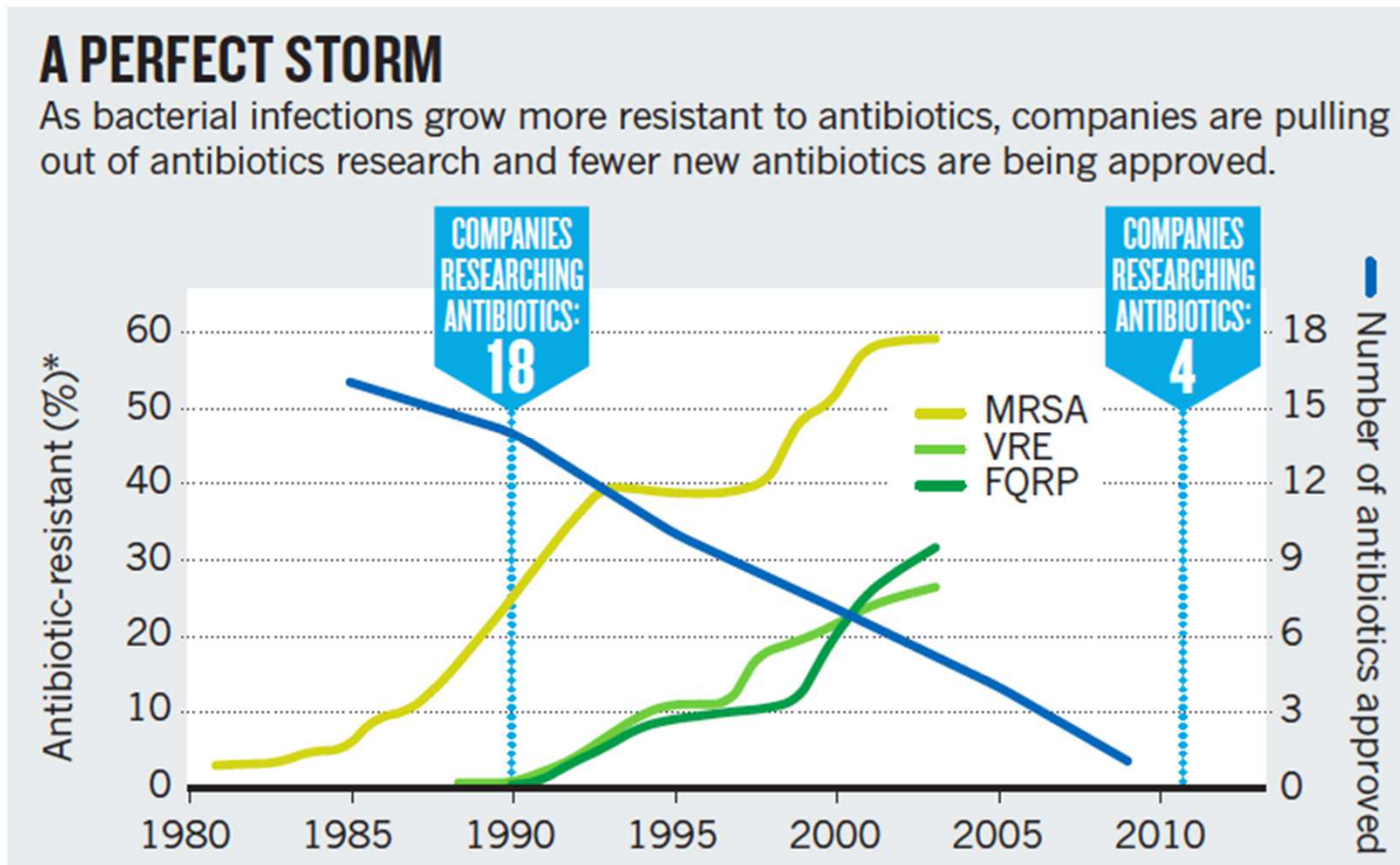
## Résistances - France et Europe

	Country	2010			2011			2012			2013		
		N	%R	(95% CI)	N	%R	(95% CI)	N	%R	(95% CI)	N	%R	(95% CI)
PIP-TAZ R	France	1125	20.3	(18-23)	1572	22.5	(20-25)	1627	19.9	(18-22)	1815	15.4	(14-17)
	EU/EEA (population-weighted mean)		15.7	(13-19)		16.2	(13-20)		16.6	(14-20)		16.2	(14-19)
FQ R	France	1181	22.8	(20-25)	1554	27.0	(25-29)	1723	22.2	(20-24)	1863	21.2	(19-23)
	EU/EEA (population-weighted mean)		21.7	(18-25)		22.1	(19-26)		20.8	(18-24)		20.0	(17-23)
Cefta R	France	1009	12.7	(11-15)	1466	16.0	(14-18)	1607	14.1	(12-16)	1868	11.5	(10-13)
	EU/EEA (population-weighted mean)		11.9	(9-15)		12.9	(10-16)		13.4	(11-16)		12.2	(10-15)
Aminosides R	France	1138	18.4	(16-21)	1599	21.3	(19-23)	1259	24.5	(22-27)	1869	15.5	(14-17)
	EU/EEA (population-weighted mean)		16.5	(14-20)		17.0	(14-20)		18.5	(16-22)		15.9	(13-19)
Carbapénèmes R	France	1186	17.8	(16-20)	1622	20.0	(18-22)	1722	18.0	(16-20)	1862	17.2	(15-19)
	EU/EEA (population-weighted mean)		17.0	(14-20)		16.9	(14-20)		17.1	(14-20)		17.6	(14-20)

European Antimicrobial resistance surveillance network (Ears-Net) 2013

# Problématique

## Appauvrissement de la filière R&D antibiotique

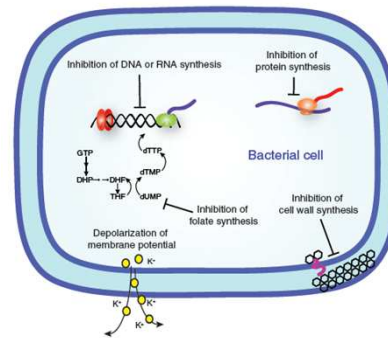


# Problématique

## Adjuvants aux antibiotiques

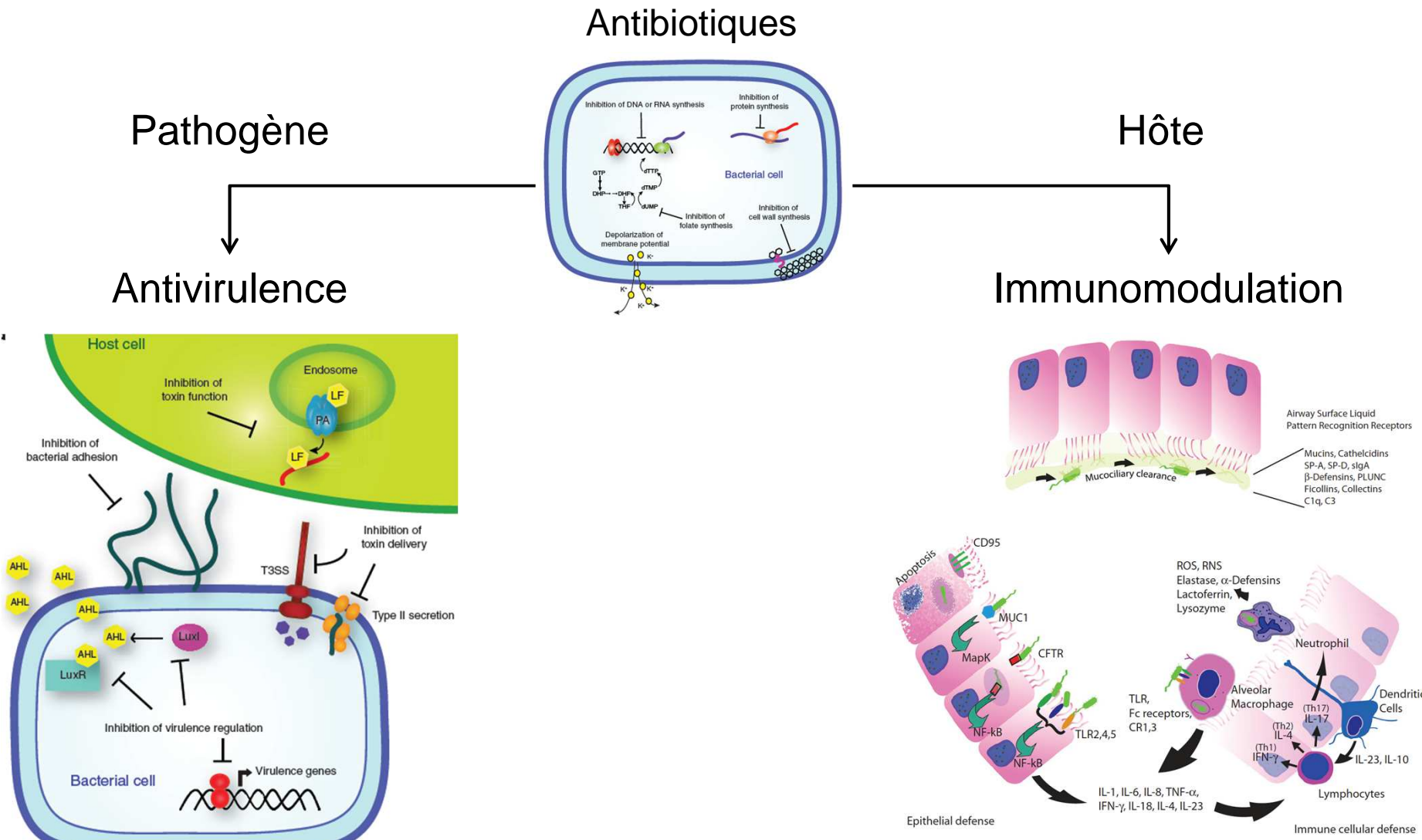
---

### Antibiotiques



# Problématique

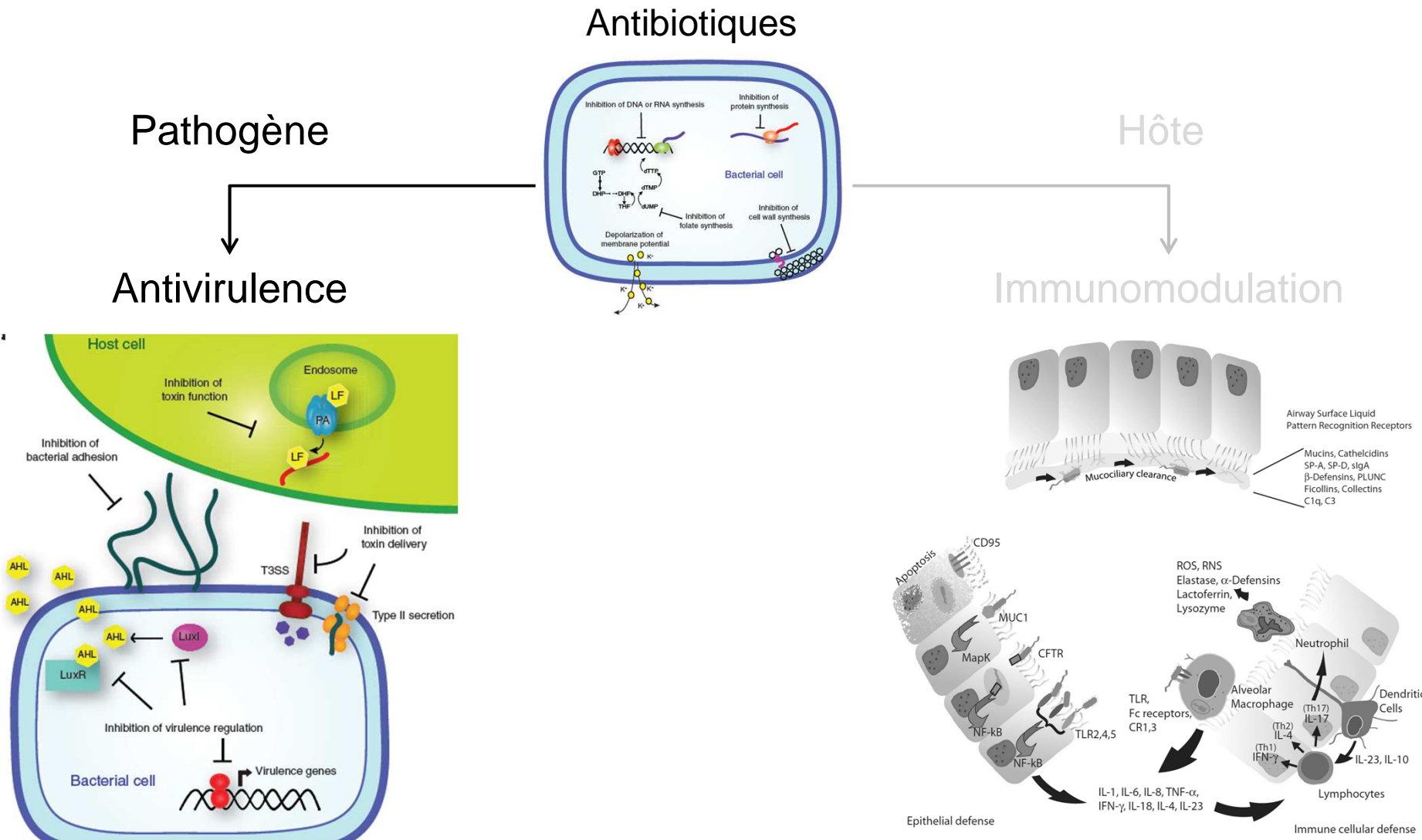
## Adjuvants aux antibiotiques





# Problématique

## Adjuvants aux antibiotiques

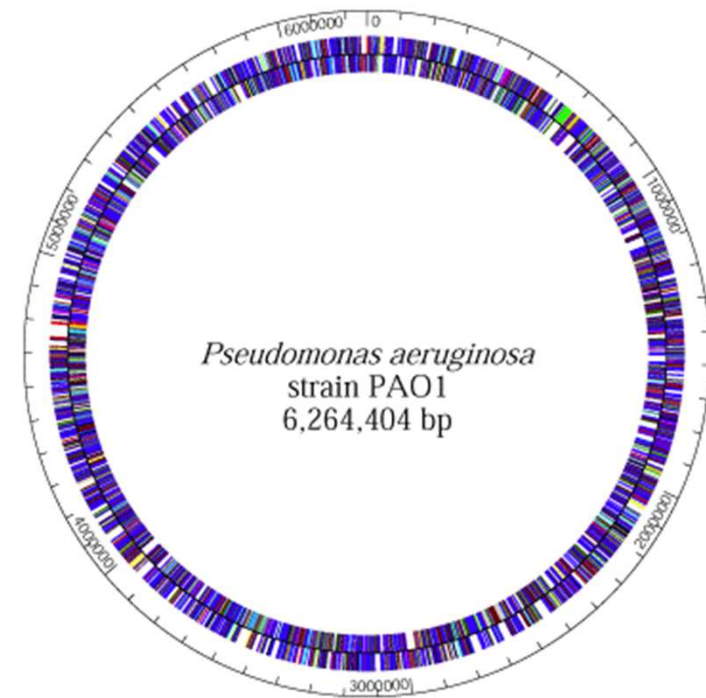


# Virulence

## Génomome et virulence

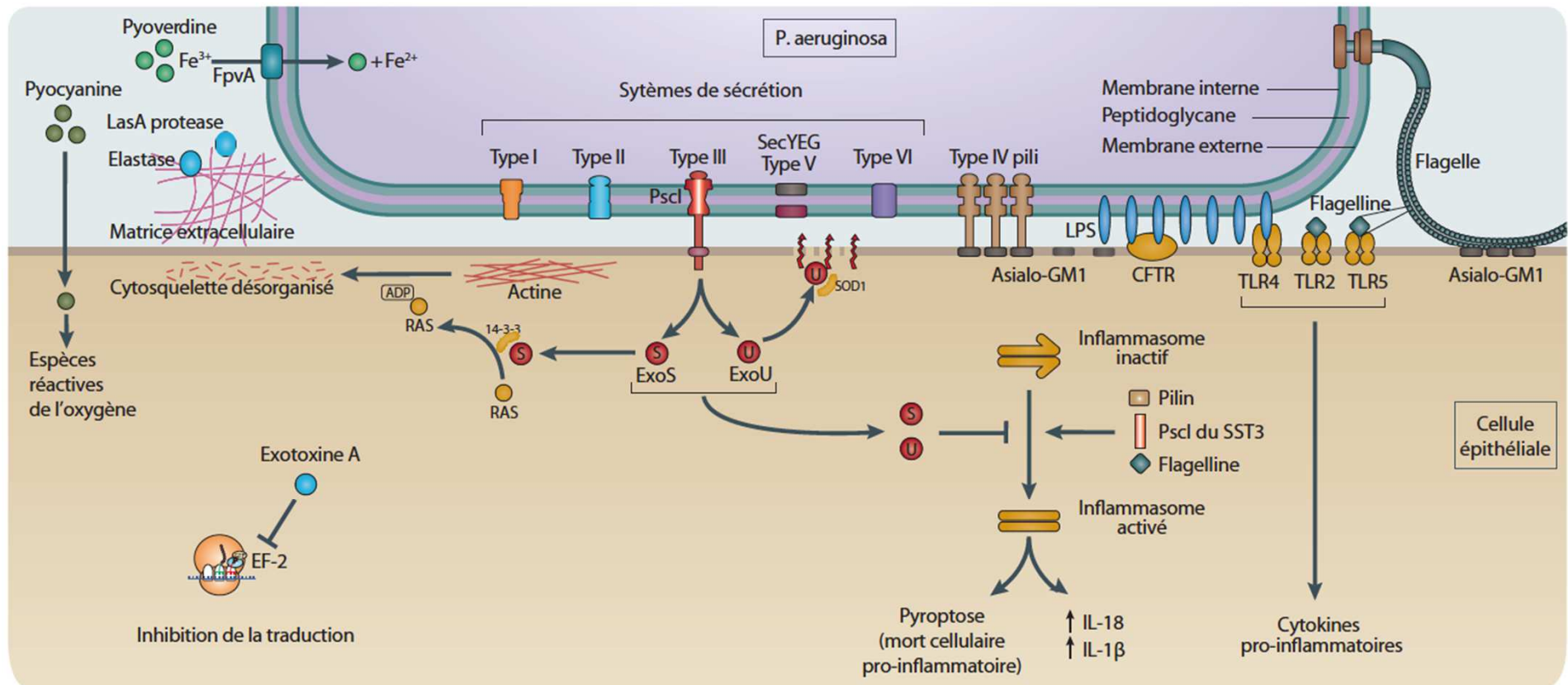
---

- Gros génome > 6.3 M pdb
- Nombreux gènes de virulence
  - adhésines,
  - transport et de translocation de facteurs de virulence,
  - pompes d'efflux d'antibiotiques
  - détecteurs de l'environnement
- 260 gènes de virulence conservés
- + gènes de virulence concentrés dans des *îlots de pathogénicité*



# Virulence(s)

## Arsenal de facteurs de virulence

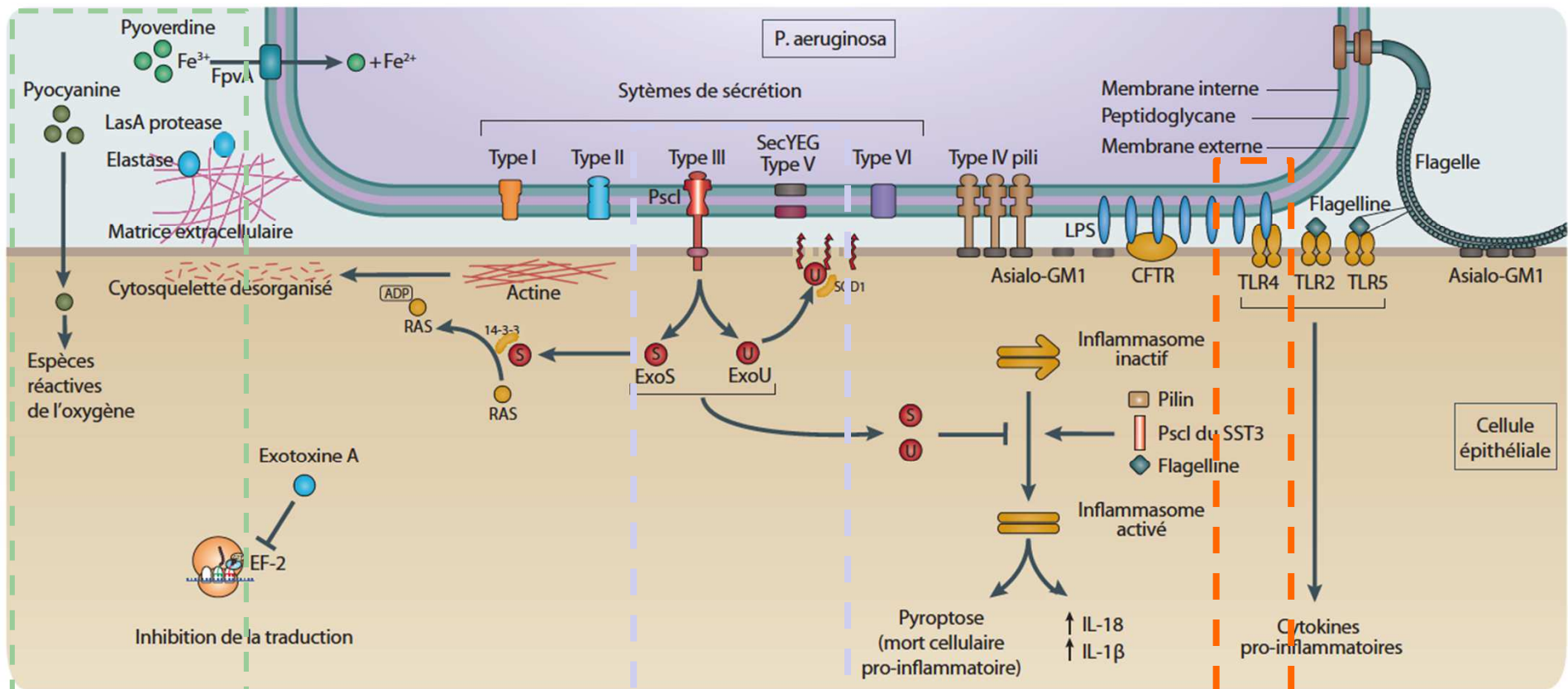


modifié à partir de Hauser Nat Rev Microbiol 2011

Modifié à partir de Hauser Nat Rev Microbiol 2011

# Virulence(s)

## Arsenal de facteurs de virulence



**Quorum-sensing dependants**  
(communication intercellulaire)  
Pyocyanine  
Elastase

**SST3**  
"injection" de toxines

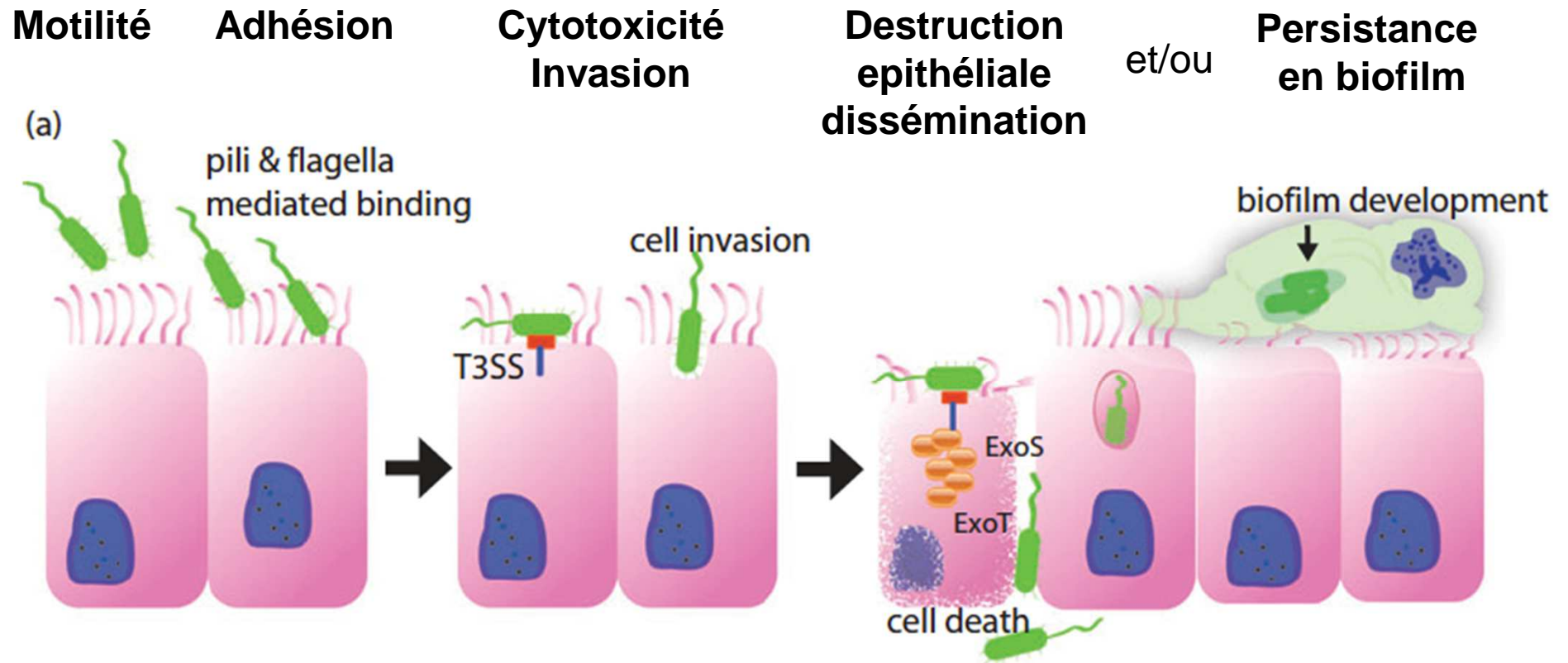
**LPS / Flagelle**  
adhésion  
inflammation

**Adhésines**  
Pili  
**Lectines**



# Virulence(s)

## Adhésion et virulence



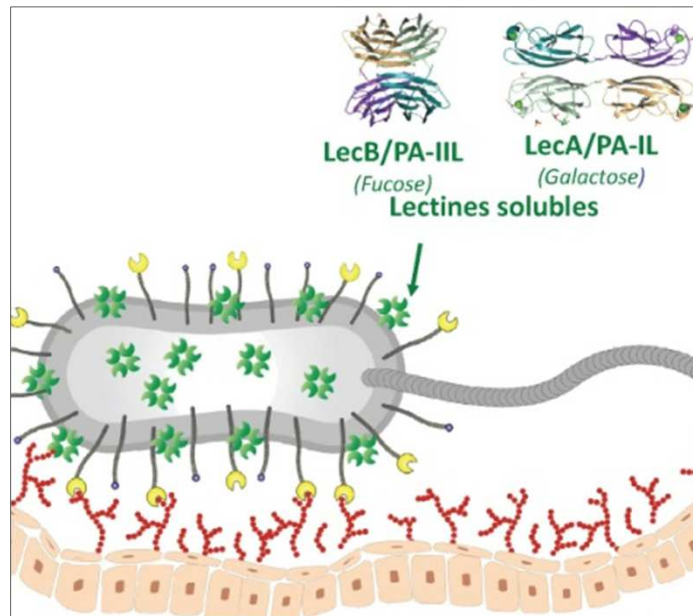
# Cibles et voies thérapeutiques

---

*Lectines de P. aurignosa*

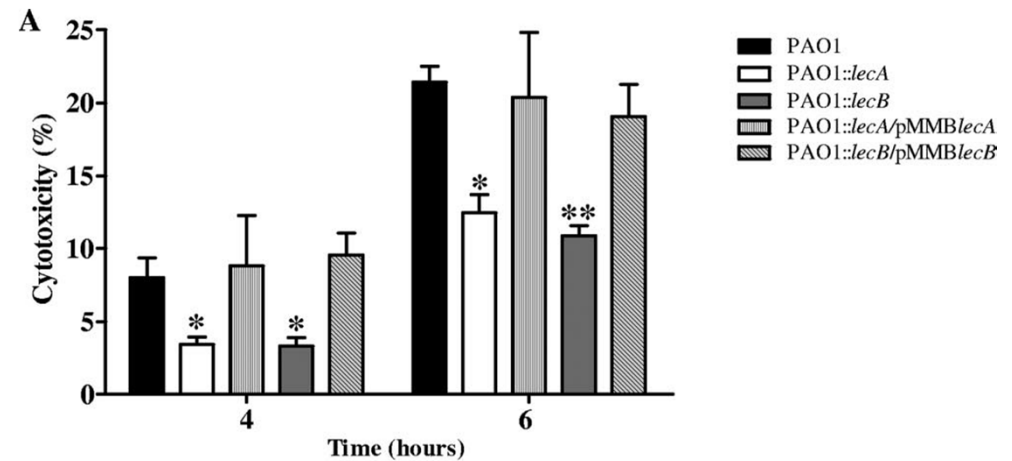
# Lectines

## Lectines et adhésion *P. aeruginosa*



- **Adhésines**

- secrétées par *P. aeruginosa*
- sous la dépendance du QS
- adhèrent aux sucres de paroi



### Cytotoxicité directe sur A549

- souches productrices de lectines
- lectines solubles elle-mêmes

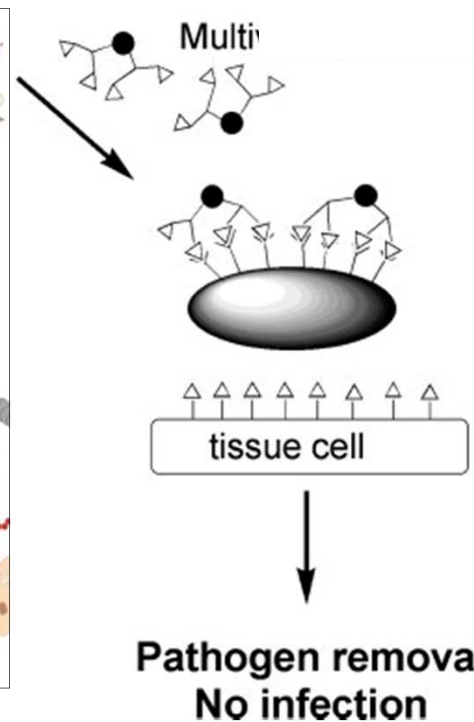
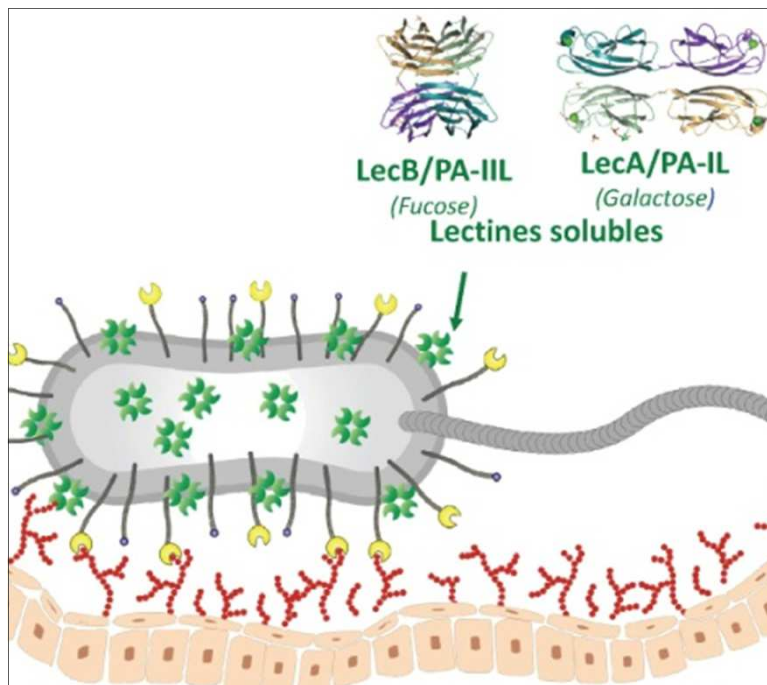


# Lectines

## Inhibiteurs d'adhésion : les glycomolécules

**Cible : lectines**

**Inhibiteurs = glycomolécules**



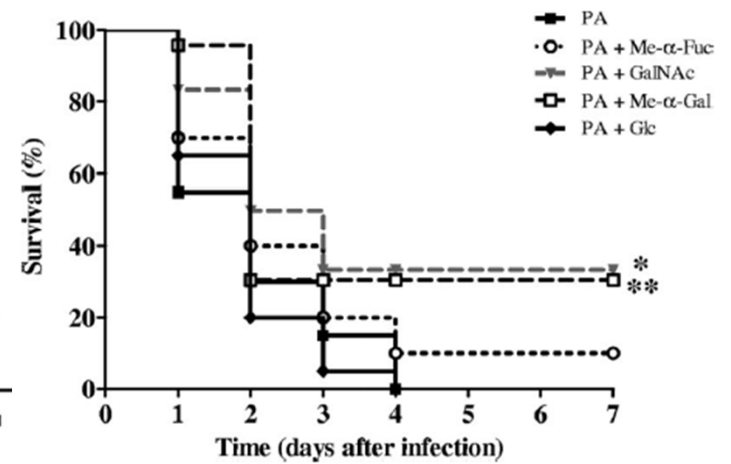
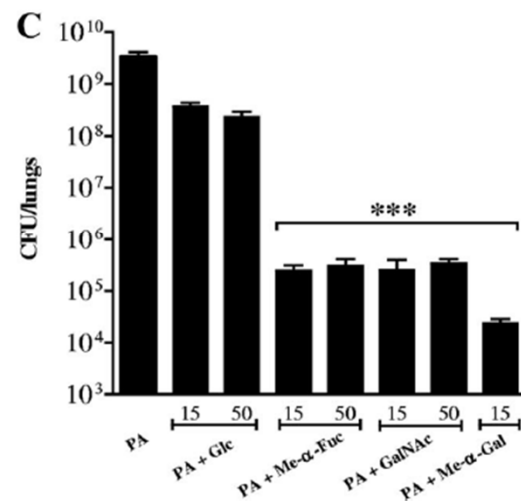
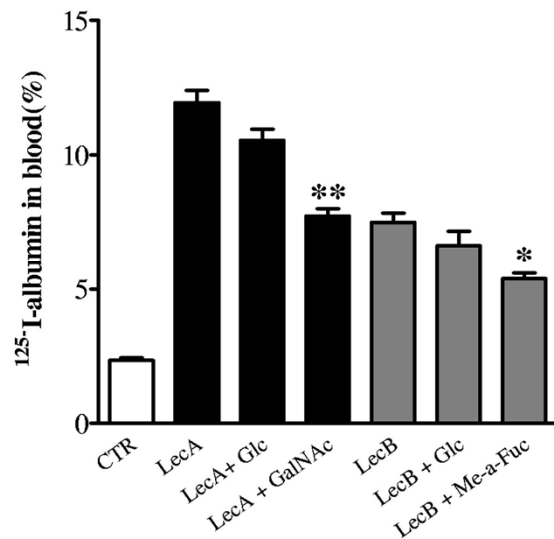
- Sucres simples
  - Fucose/galactose
- Complexes
  - GalMe/MeFuc
- Glycomimétiques
- Glycoclusters

# Lectines

## Inhibiteurs de lectines : saccharides

Modèle murin de pneumonie létale

Co-instillation inhibiteurs = dérivés glycosidiques de sucres simples



# Inhibiteurs de lectines

## Glycomolécules inhibiteurs de lectines

---

- Sucres simples
  - galactose
  - fucose



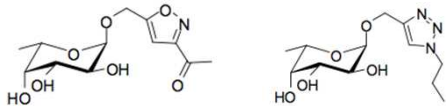
- adhésion in-vitro : 10-40%
- lésion in-vivo : 20-25%
- charge in-vivo : 1 log CFU/ml

- Dérivés
  - N Acetylés
  - $\alpha$  méthylés



- adhésion in-vitro : 10-50%
- lésion in-vivo : 20-30%
- charge : 1-2 log CFU/ml

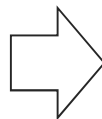
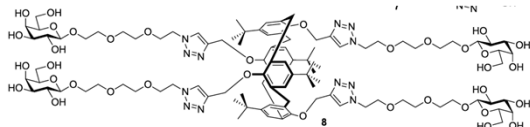
- Glycomimétiques



- adhésion in-vitro : 70-80%
- lésion in-vivo : 20-30%
- charge in-vivo : 1-2 log CFU/ml

Chemani Infect Immunity (+ données non-publiés)

- Glycoclusters



- adhésion in-vitro : **> 90%**
- lésion in-vivo : **80-100%**
- charge in-vivo : **3-4 log CFU/ml**

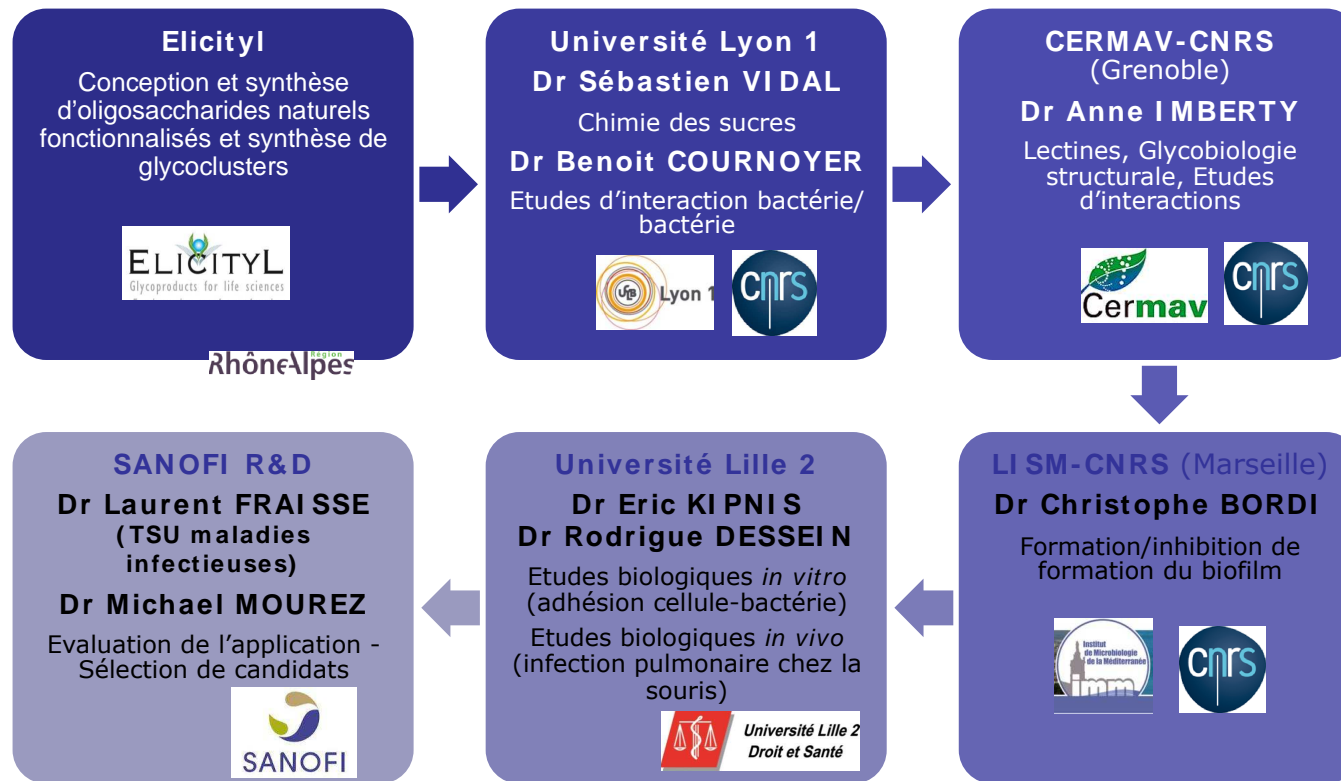
# Inhibiteurs de lectines

## Anti-Pyo : 14eme AAP Fonds Unitaire Interministériel

### glycoclusters inhibiteurs de lectines

#### 2 industriels

#### 4 partenaires académiques à l'origine du brevet initial



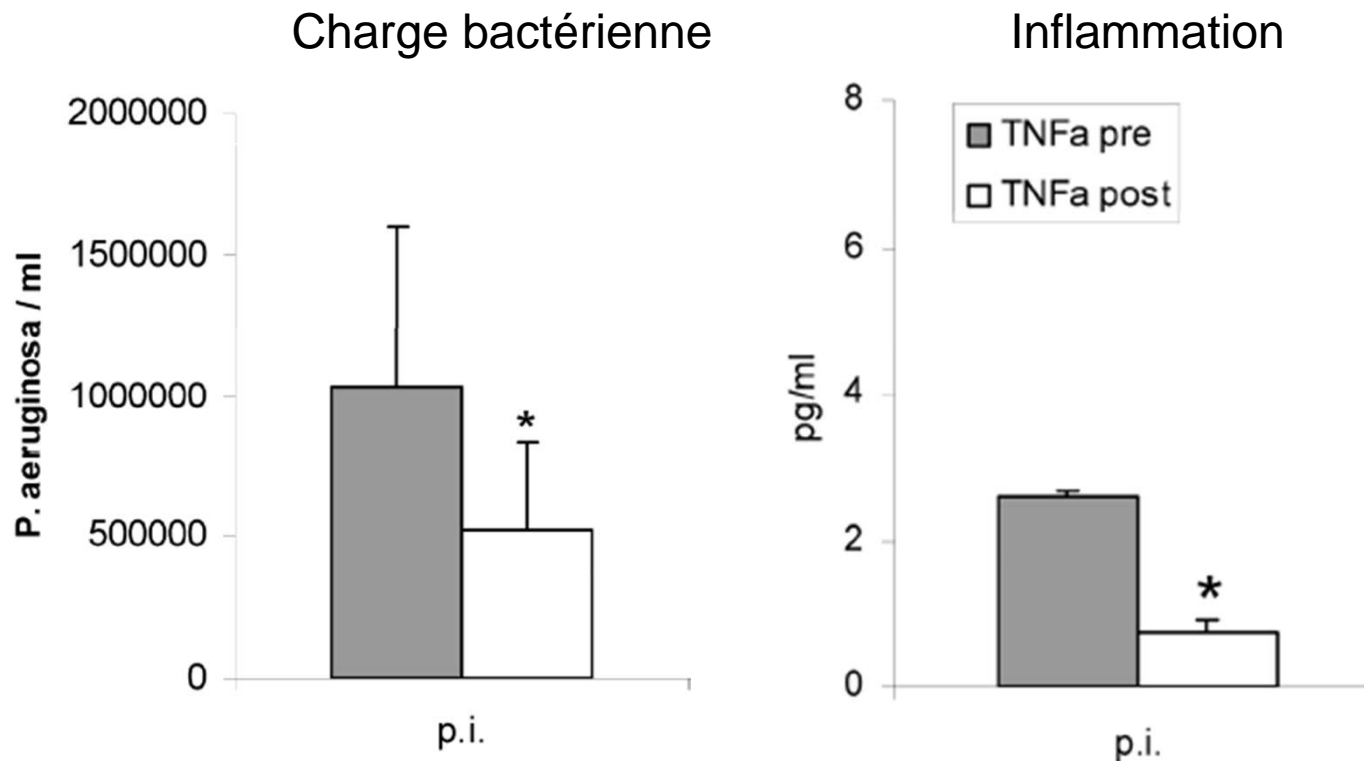
# inhibiteurs de lectines

## Inhibiteurs de lectines en clinique

---

### sucres « simples »

- 10 **patients** atteints de mucoviscidose
- fucose/galactose inhalés pendant 21j



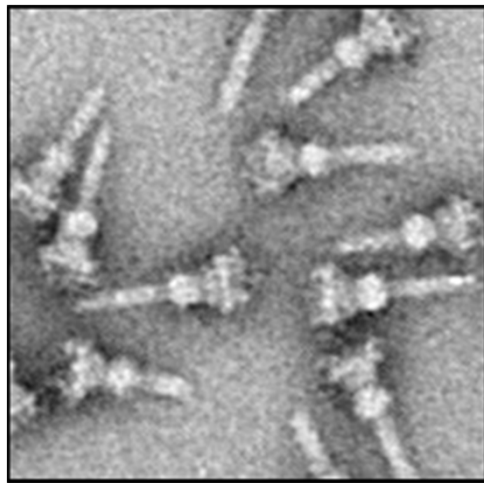
# Cibles et voies thérapeutiques

---

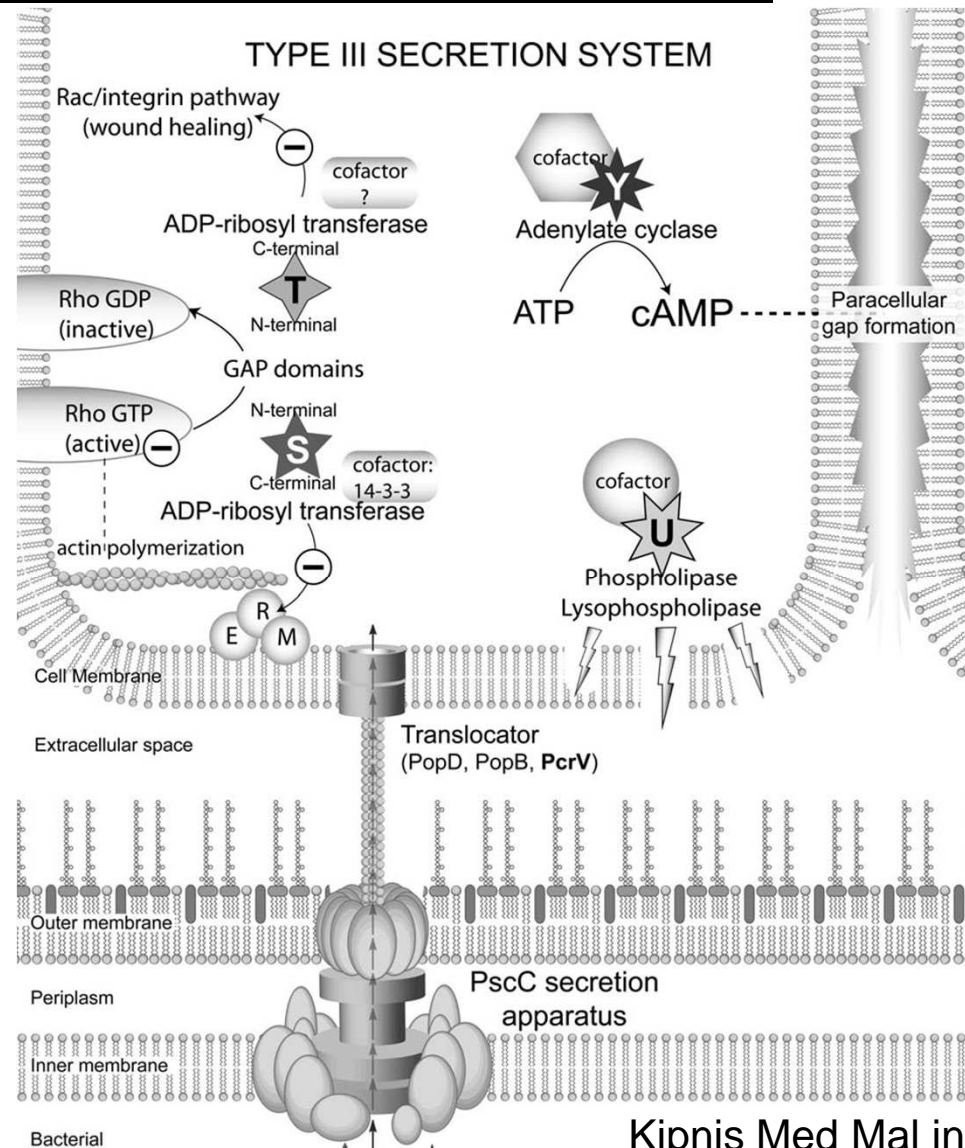
*Système de Sécrétion Type 3 (SST3)*

# SST3

## Translocation d'exotoxines dans la cellule eucaryote



Filloux Front Microbio 2011

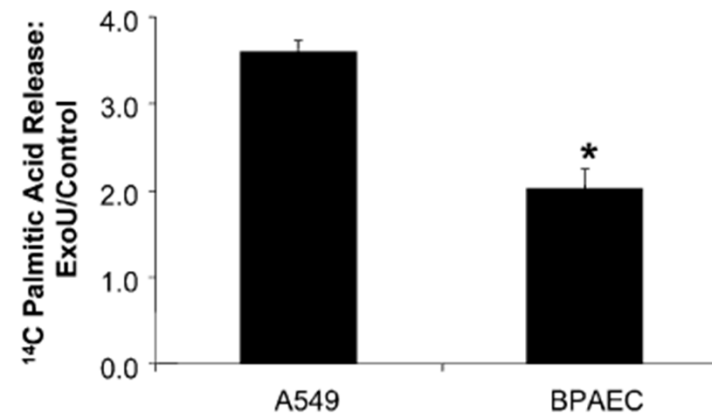
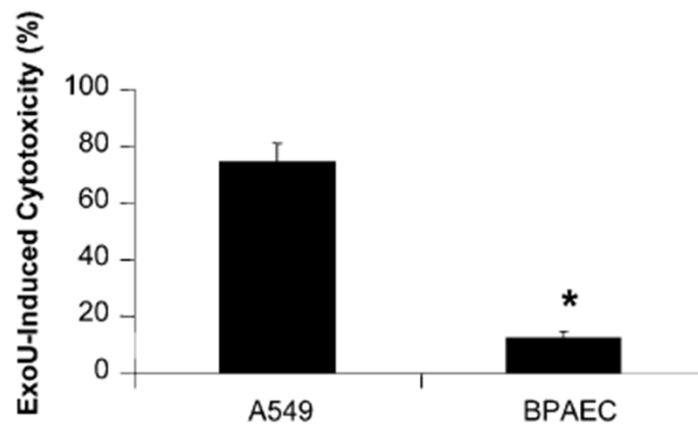
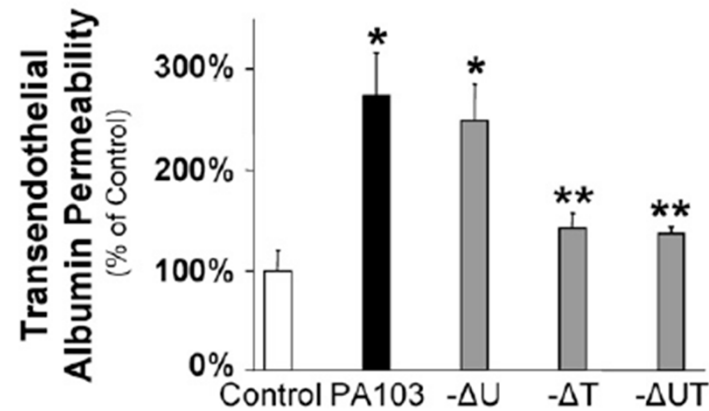


Kipnis Med Mal inf 2006

# SST3

## Pathogénicité *in-vitro* ~ SST3

ExoU cible les cellules *épithéliales* pulmonaires



Nguyen ATS 2005

Ganter AJRCMB 2009



# SST3

## Pathogénicité *in-vivo* ~ SST3

---

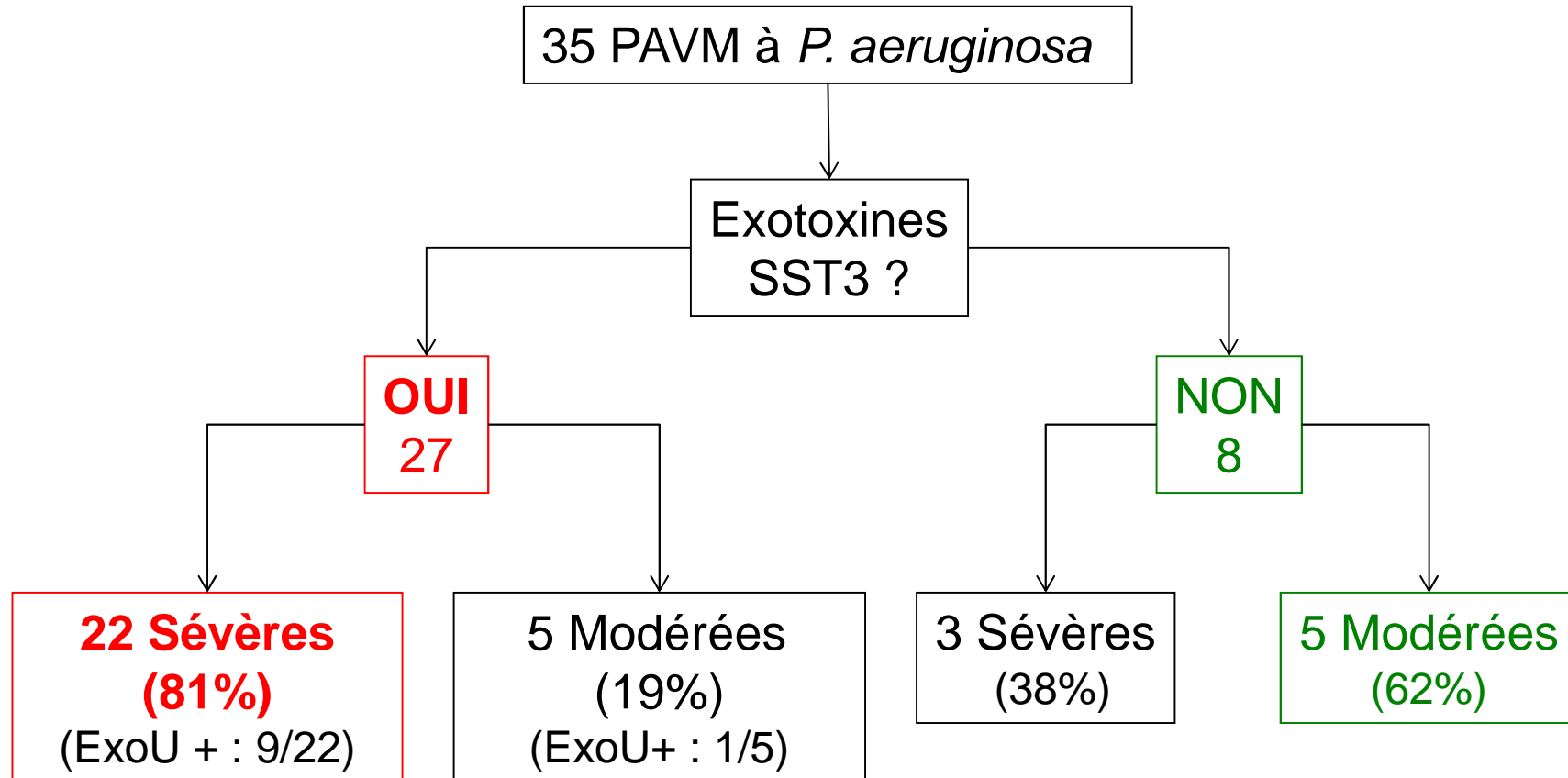
58 souches non clonales de patients atteints de PAVM  
Réinstillées dans un modèle murin de pneumonie

---

	Percentage of Lung Protein Tracer in the Blood Mean (95% CI)	Lung Wet to Dry Weight Ratio Mean (95% CI)	Bacteremia Odds Ratio (95% CI)
Type III secretion system	$p < .0001$	$p < .0001$	$p = .026$
Nonsecreting	4.41 (3.54–5.15)	4.27 (4.14–4.40)	1
Exo ST	6.78 <sup>a</sup> (5.76–7.51)	4.44 (4.32–4.56)	2.19 (0.48–10.05)
Exo U	12.82 <sup>a</sup> (11.26–14.15)	5.22 <sup>a</sup> (5.02–5.37)	9.00 <sup>a</sup> (1.72–46.99)

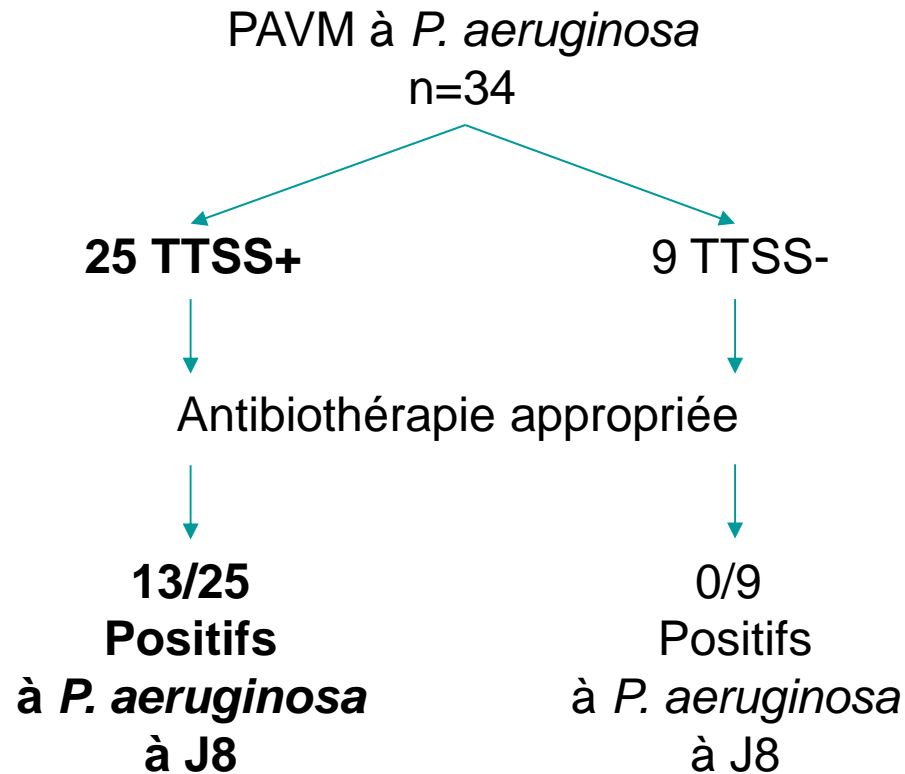
# Pathogénicité clinique ~ SST3

---

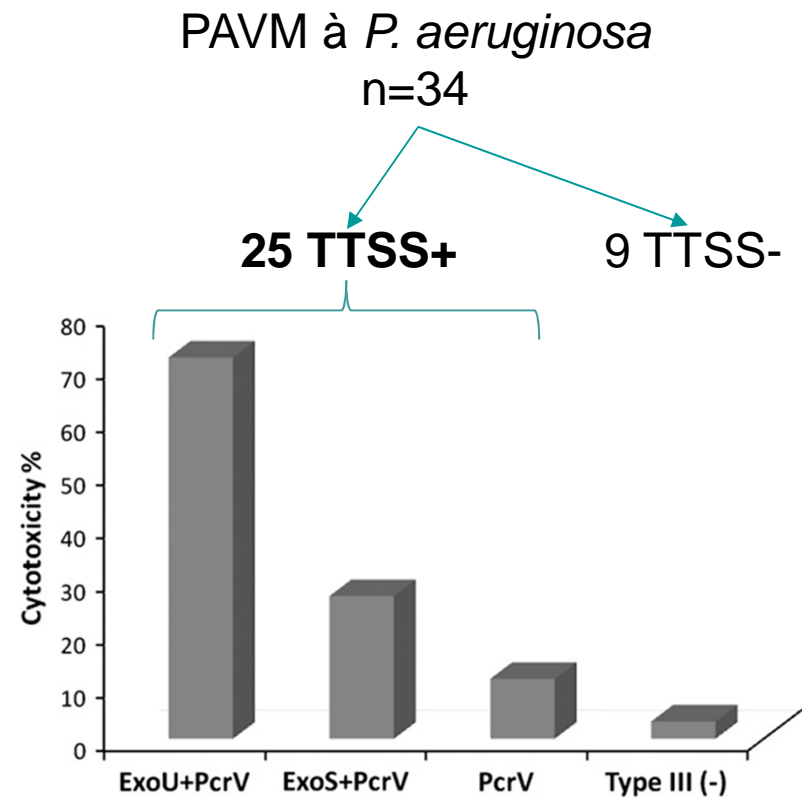


# Pathogénicité clinique ~ SST3

## Virulence clinique des isolats



## Cytotoxicité in-vitro des isolats



# Anti-SST3

## Anticorps anti-SST3 = Mab anti-PcrV

---

Exotoxines « injectées »

**Exo U**

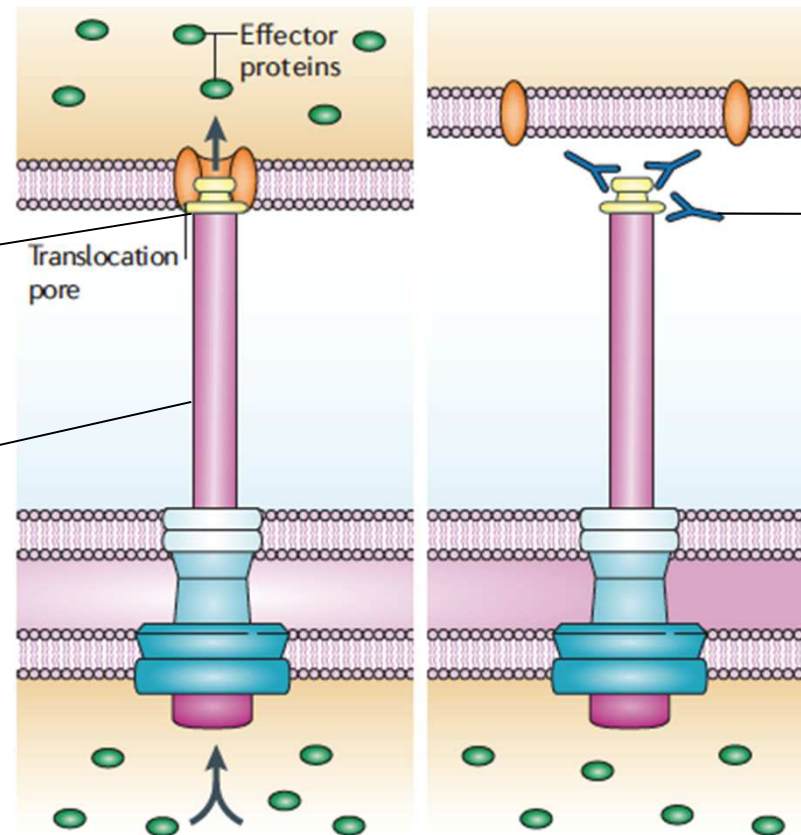
Exo S, Exo T, Exo Y

Translocon

**PcrV**

PopB/PopD

« aiguille » du SST3

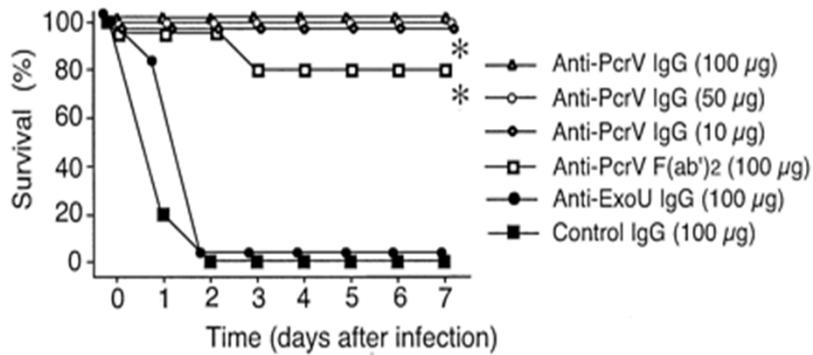


Anticorps **Anti-PcrV**

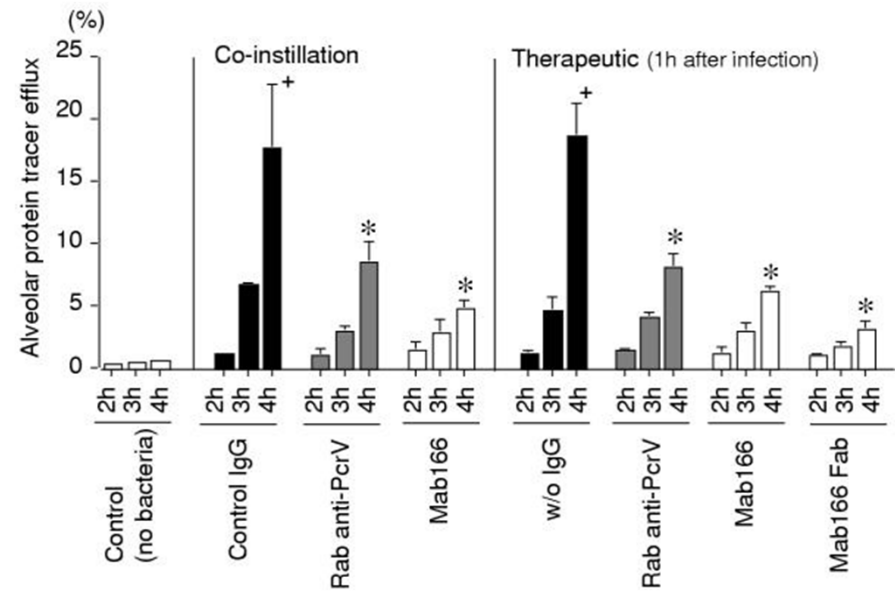
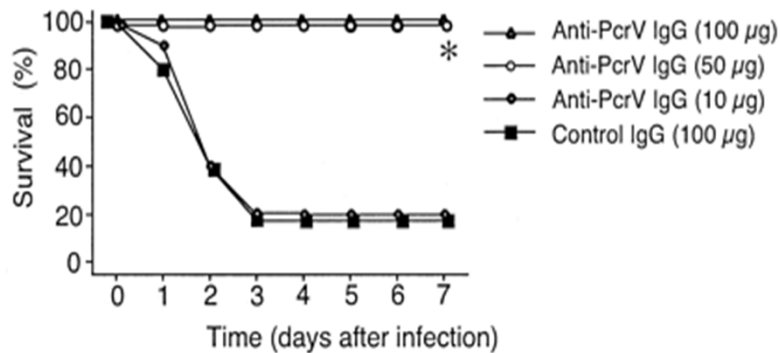
# Anti-SST3

## Mab anti-PcrV

a. Intravenous treatment 1 h after infection



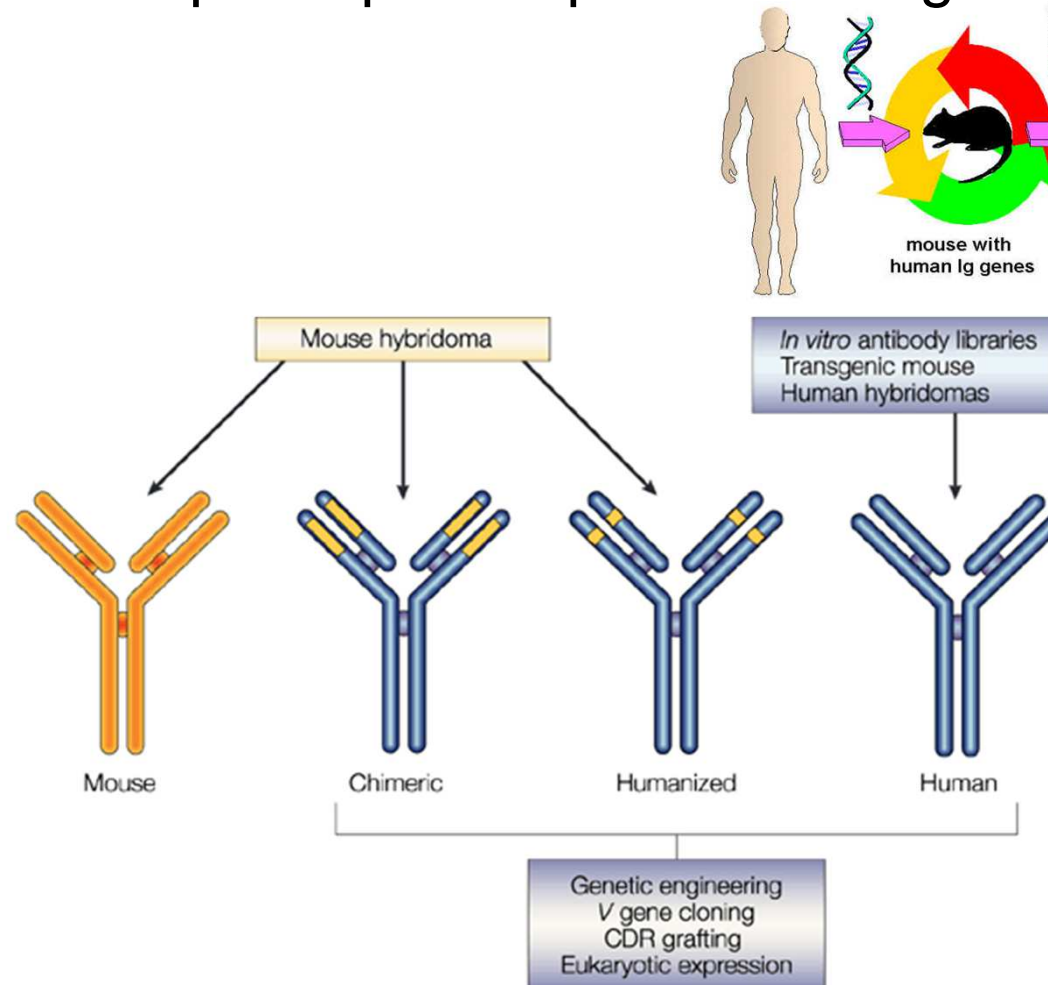
b. Intravenous treatment 4 h after infection



# Anti-SST3

## Anticorps monoclonaux « optimisés »

= spécifiques et peu immunogènes



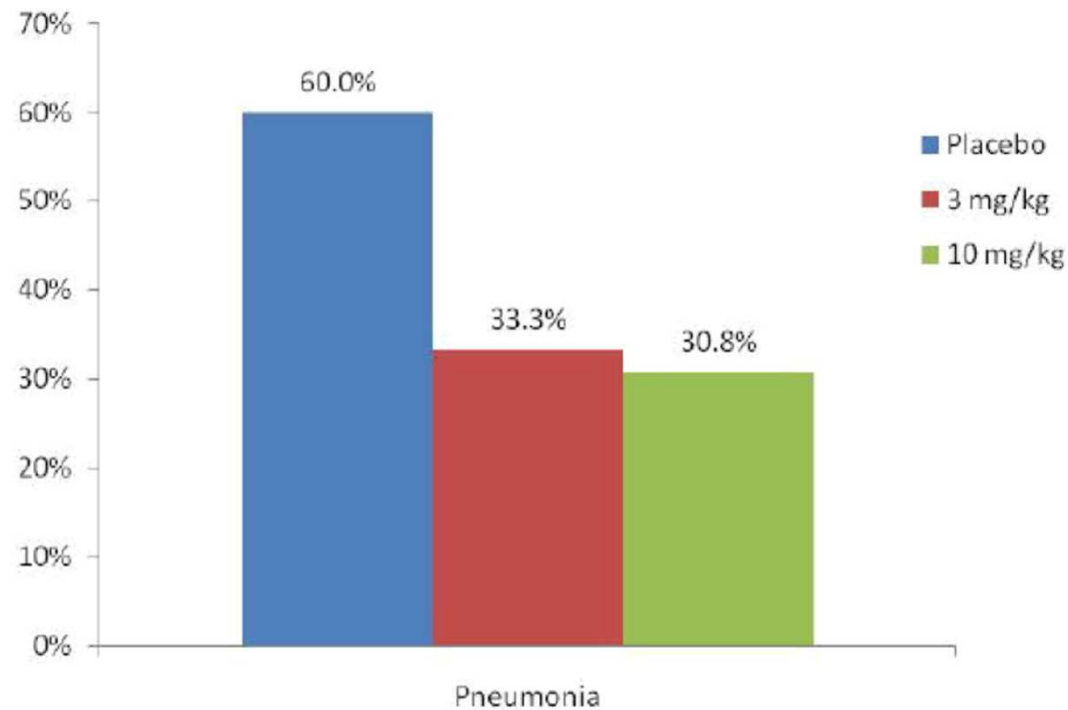
Modifié d'après Brekke Nat Rev Drug Disc 2003

# Anti-SST3

## Anticorps monoclonal humanisé anti-PcrV

### essai KB001 (Kalobios)

Essai Phase I/II chez 48 patients intubés/ventilés colonisés par *P. aeruginosa*  
prévention des pneumonies acquises sous ventilation

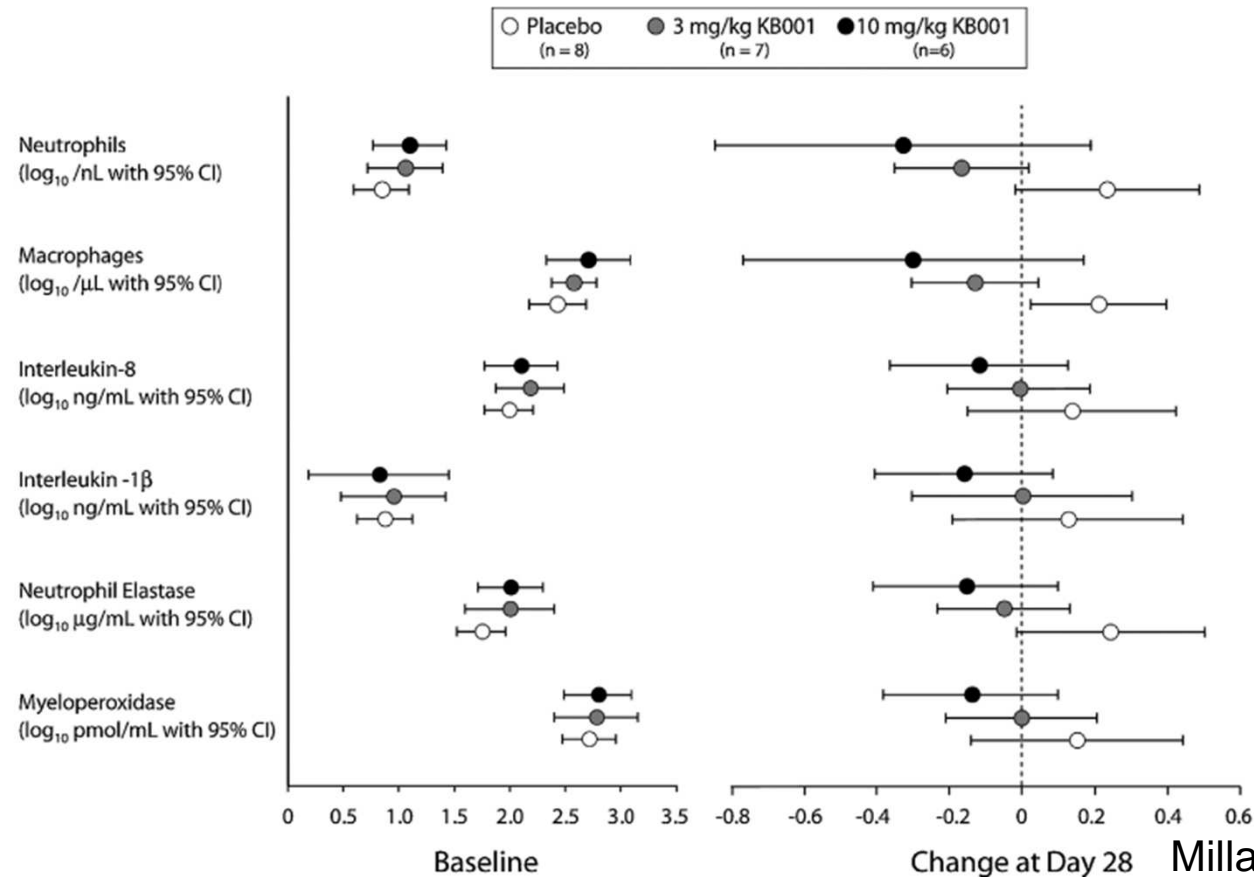


# Anti-SST3

## Anticorps monoclonal humanisé anti-PcrV

### essai KB002 (Kalobios)

Essai Phase I/II chez 12 patients muco colonisés par *P. aeruginosa*  
*impact sur inflammation et symptômes*





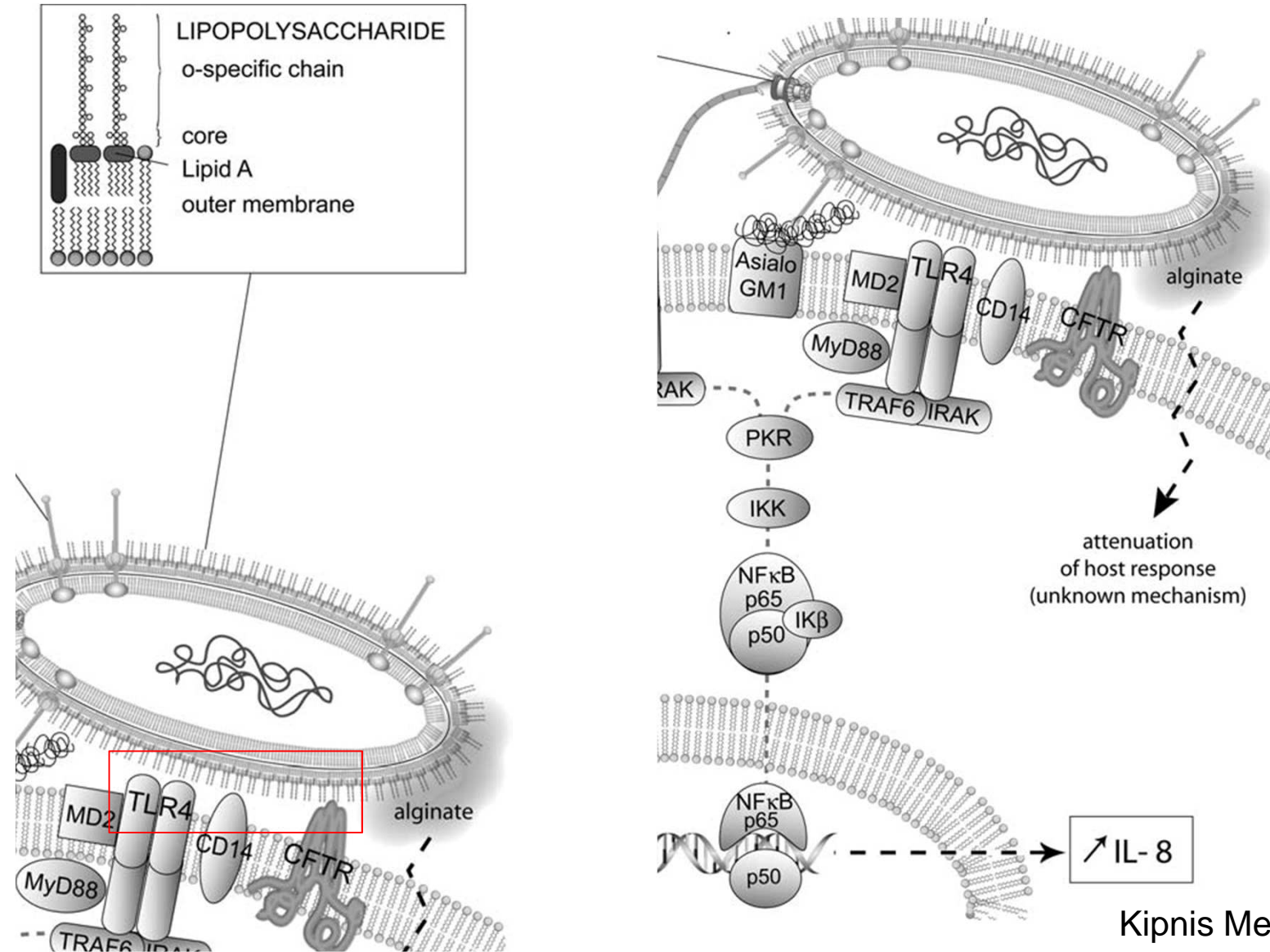
# Cibles et voies thérapeutiques

---

*Lipopolysaccharide (LPS)*

# LPS

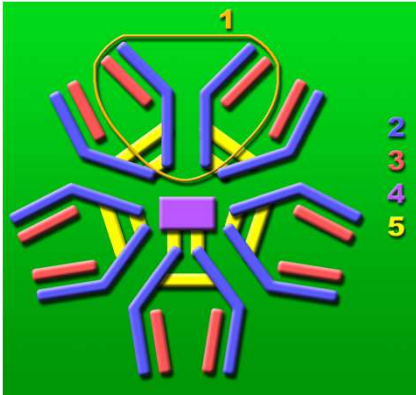
## Lipopolysaccharide



# LPS

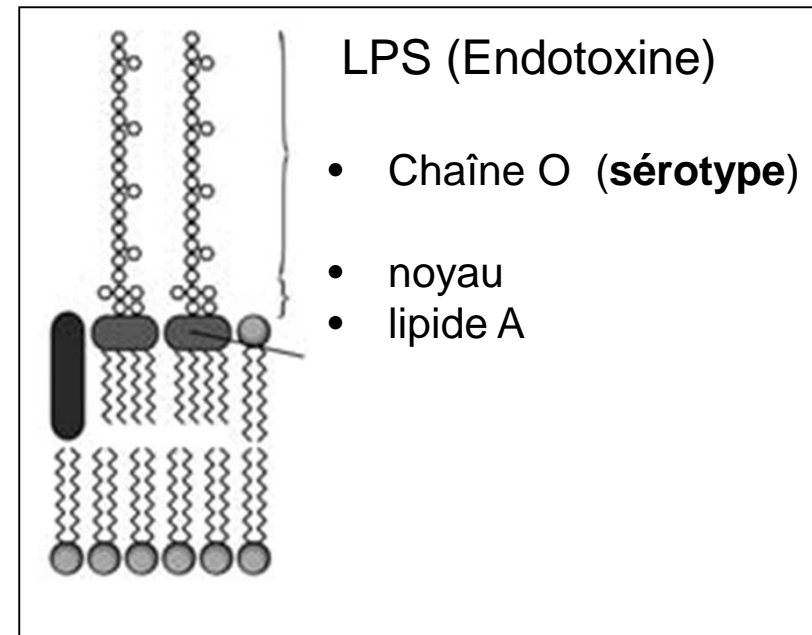
## Anti-antigène O11 du Lipopolysaccharide

Anticorps  
Panobacumab  
(Aerumab11<sup>®</sup>, Kenta Biotech)



- 1: Unité de base (humaine)
- 2: Chaînes lourdes (humaines)
- 3: Chaînes légères (humaines)
- 4: Chaîne J anti-O (souris)**
- 5: Ponts disulfure

Cible



# LPS

## Prévalence sérotype O11 et PAVM

---

56 souches cliniques de PAVM (étude Pyopneumagène)

---

Lipopolysaccharide Phenotype	O Antigen	Serotype
Non typeable		n = 21
O6		n = 13
O11		n = 10
O3		n = 3
O4		n = 3
O12		n = 2
O1		n = 1
O2		n = 1
O10		n = 1
O16		n = 1
Type III Secretion System Phenotype		
ExoU		n = 15
ExoS/T		n = 24
Nonsecreting		n = 18

---

# Anti-LPS

## Panobacumab anti-O11: essai phase IIa

---

---

Parameter	Safety (n=18)	ITT (n=17)	PK (n=17)	PP (n=13)
Overall patient survival at 30 days/ end of study	15 (83.3%)	14 (82.4%)	15 (88.2%)	13 (100%)
Clinical outcome				
resolution	11 (61.1%)	11 (64.7%)	11 (64.7%)	11 (84.6%)
recurrence	2 (11.1%)	2 (11.7%)	2 (11.7%)	2 (15.4%)
continuation <sup>a</sup>	5 (27.8%)	4 (23.5%)	4 (23.5%)	0 (0%)

---

### Phase II

- 18 PAVM
- 3 administrations
- Aucun EI grave
- > 80% de survie
- uniquement PAVM à SEROTYPE O11!
- Isolement/sérotypage = 48h

# Cibles et voies thérapeutiques

---

*Quorum-sensing (QS)*

# Quorum-sensing

## Quorum-sensing

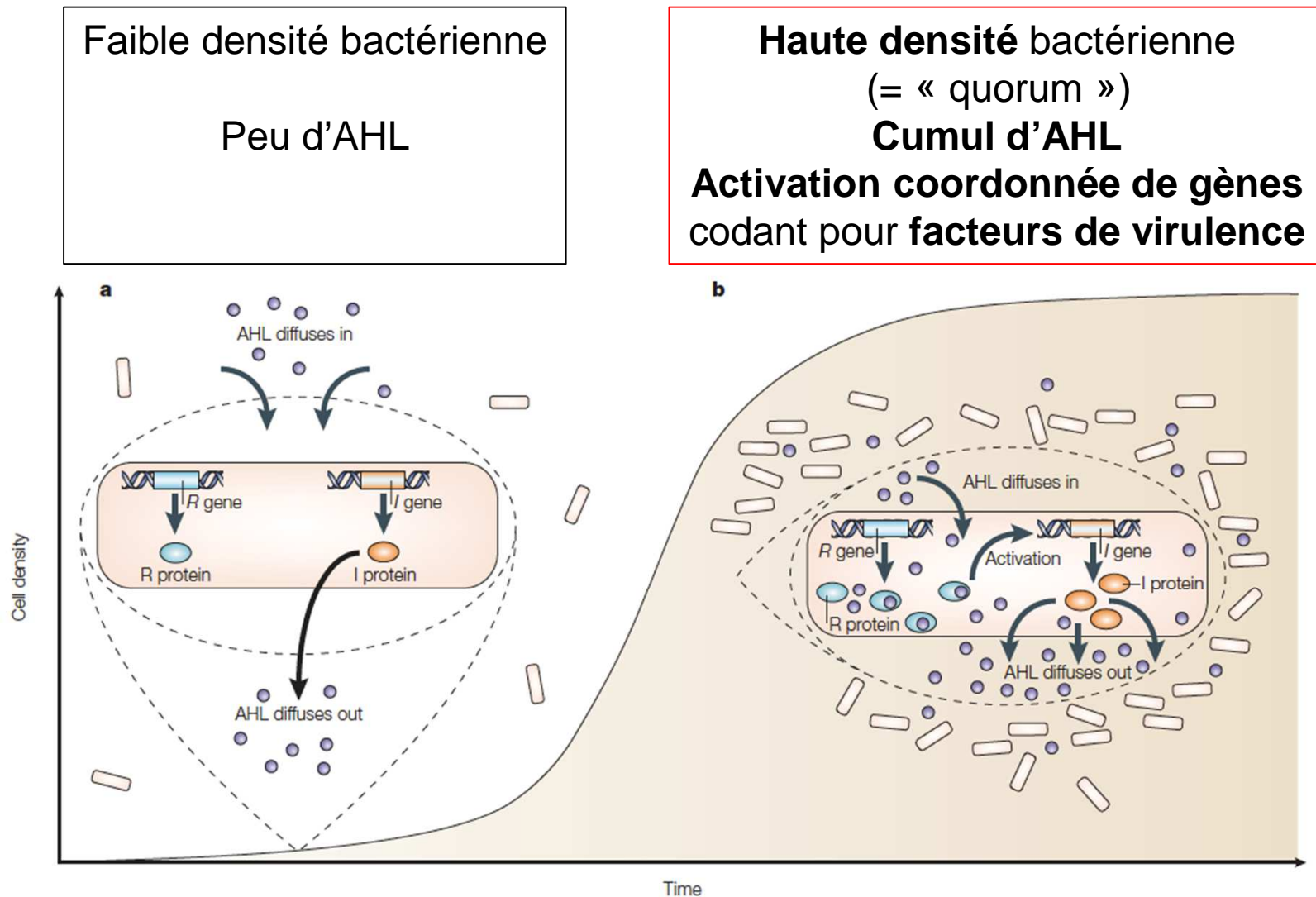


Organism	I/R genes	AHLs synthesized	QS-regulated phenotypes	
<i>Vibrio fischeri</i>	<i>luxR, luxI</i> <i>ainS</i>	3O-C6-HSL C8-HSL	Bioluminescence Colonization factors	
<i>Pantoea stewartii</i>	<i>esaR, esal</i>	3O-C6-HSL	Exopolysaccharide production	50
<i>Agrobacterium tumefaciens</i>	<i>traR, tral,</i> <i>trIR (traS)</i>	3O-C8-HSL	Virulence plasmid copy number and conjugal transfer	72
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	<i>carR, carI</i> ( <i>expR, expI</i> )*	3O-C6-HSL	Production of carbapenem antibiotic and exoenzymes	118,119
<i>Pseudomonas aeruginosa</i>	<i>lasR, lasI</i> <i>rhlR, rhlI</i> <i>qscR</i>	3O-C12-HSL C4-HSL	Virulence, biofilm formation, other cellular functions	49,53,93
<i>Rhizobium leguminosarum</i> biovar <i>viciae</i>	<i>rhlR, rhlI</i>	C6-HSL C7-HSL C8-HSL	Nodulation efficiency	15,120
	<i>raiR, rail</i>	C6-HSL C7-HSL C8-HSL	Function unknown	15,24
	<i>bisR, traR, tral</i> <i>cinR, cinI</i>	3OH-C8-HSL 3OH-C14:1-HSL	Plasmid transfer Growth inhibition	12 23

\**expI*D and *carI*D loci are highly homologous and were isolated from two different strains of *Erwinia carotovora* subsp. *carotovora* but do

# Quorum-sensing

## Communication par Acyl Homosérine Lactones

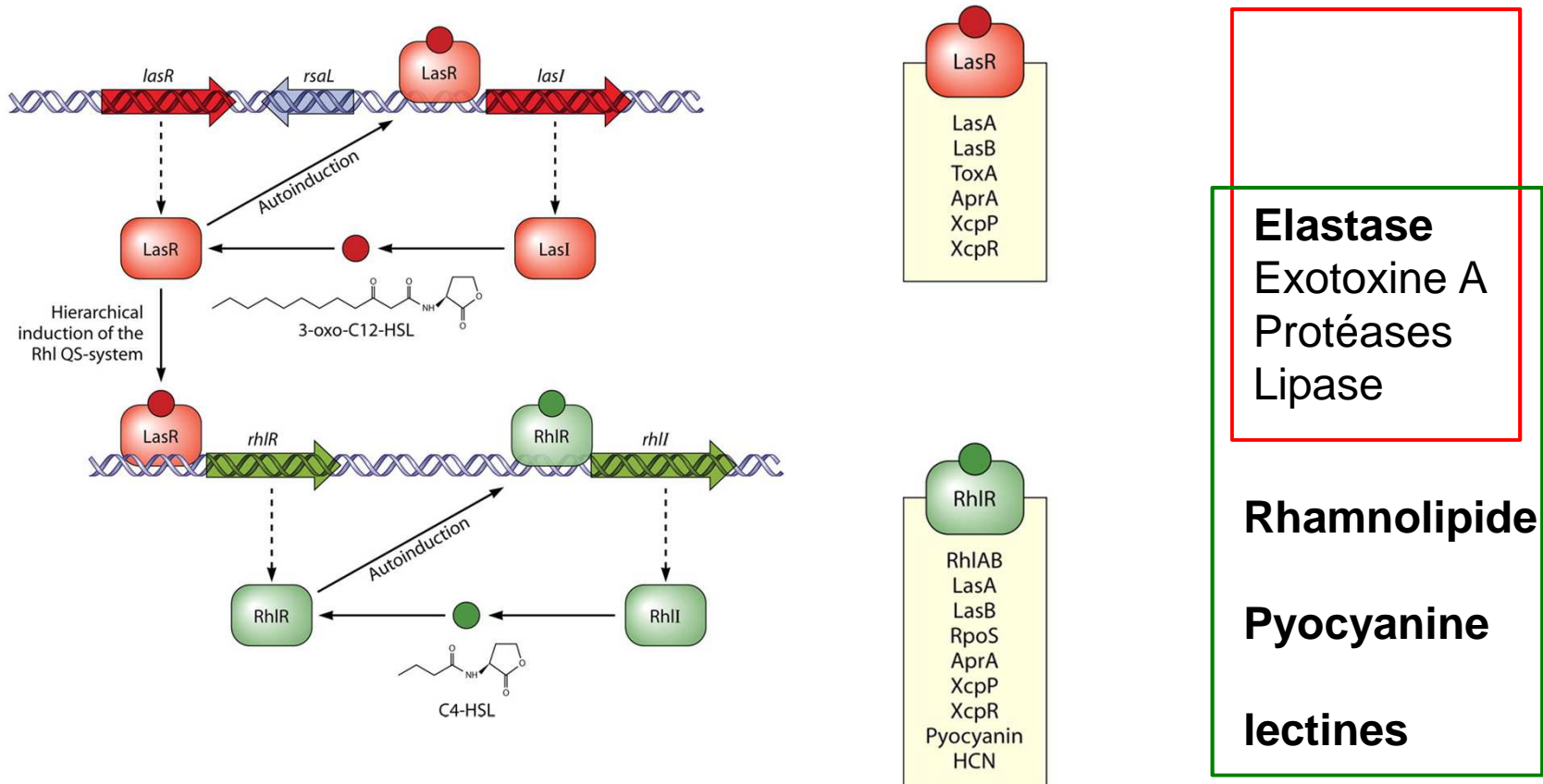




# Quorum-sensing

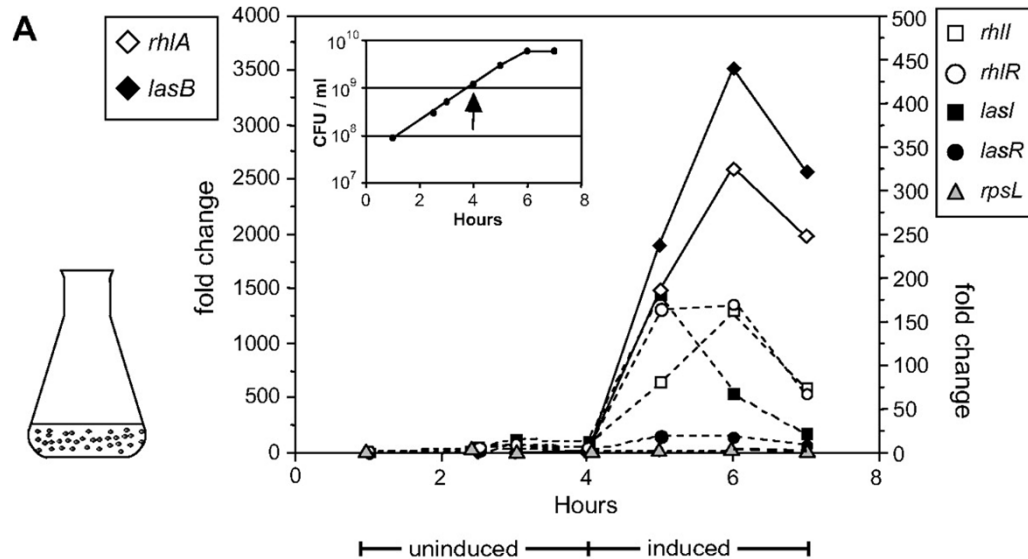
## Systèmes de QS de *P. aeruginosa*

- Système hiérarchisé
- Autoamplifié
- activent des gènes de **facteurs de virulence**

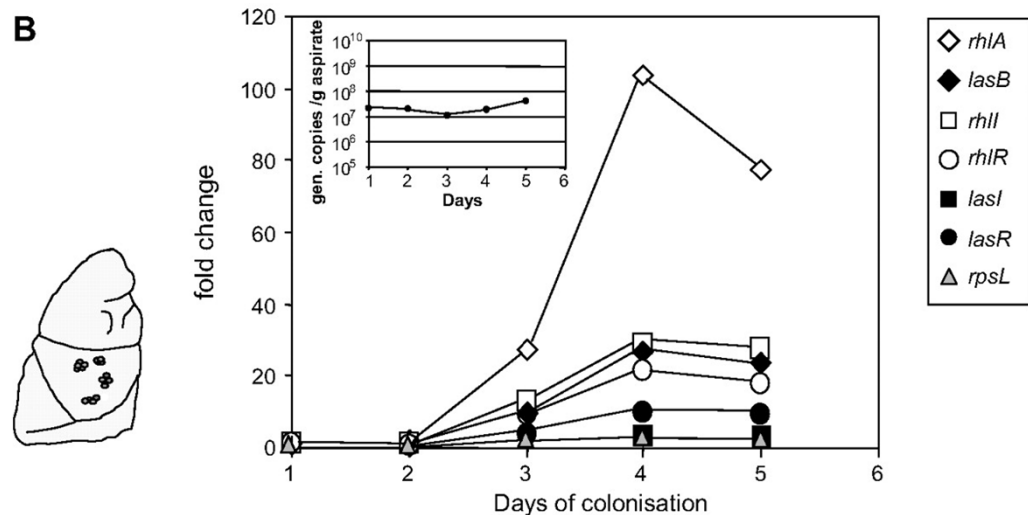


# Quorum-sensing

## Quorum-Sensing et PAVM



Profil d'expression gènes QS  
de *P. aeruginosa*  
in-vitro

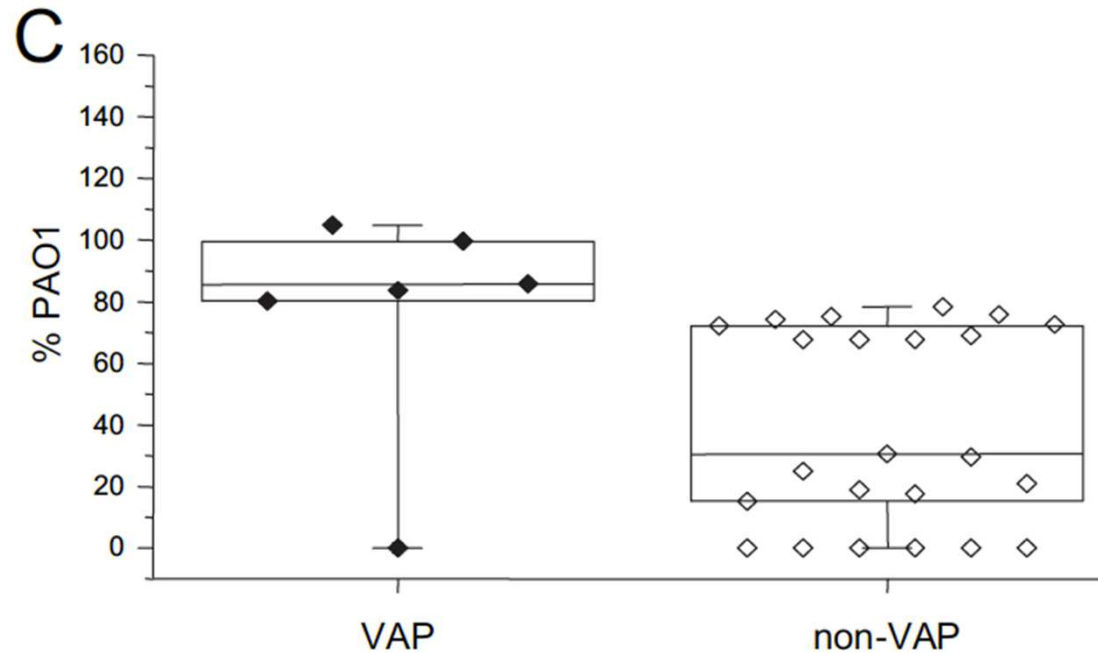


Profil d'expression gènes QS  
**chez des patients**  
colonisés par *P. aeruginosa*  
et développant une PAVM  
**= *rhlA* (rhamnolipide)**

# Quorum-sensing

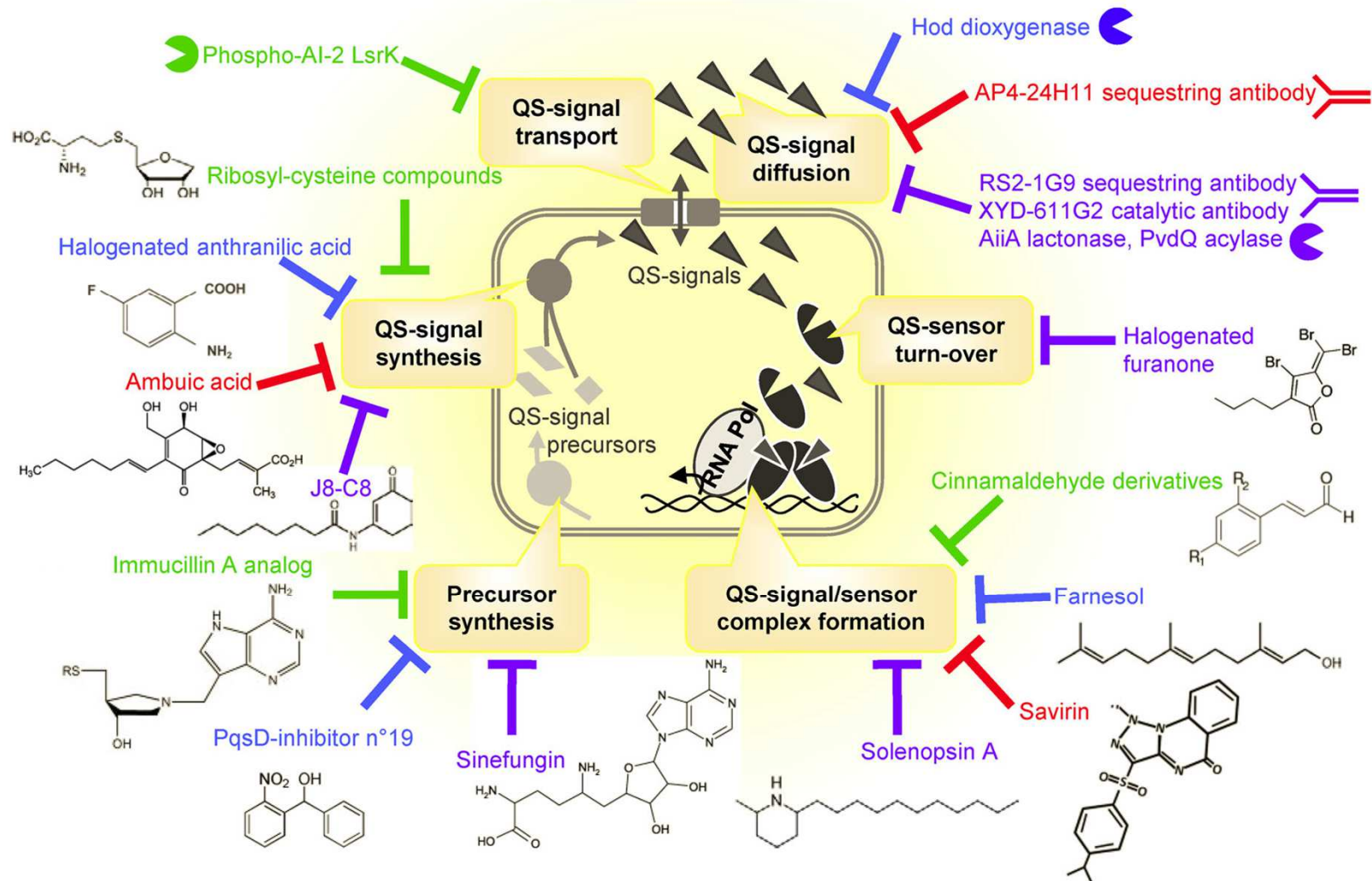
## Quorum-Sensing et PAVM

Rhamnolipide (facteur de virulence QS-dépendant)  
chez des patients colonisés par *P. aeruginosa*  
Et ayant développé une PAVM ou non



# Inhibition QS

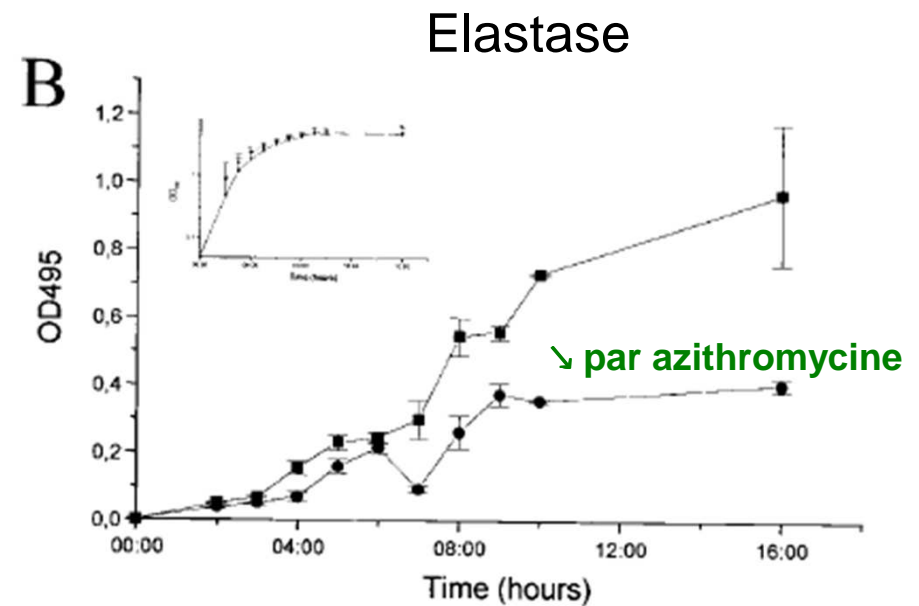
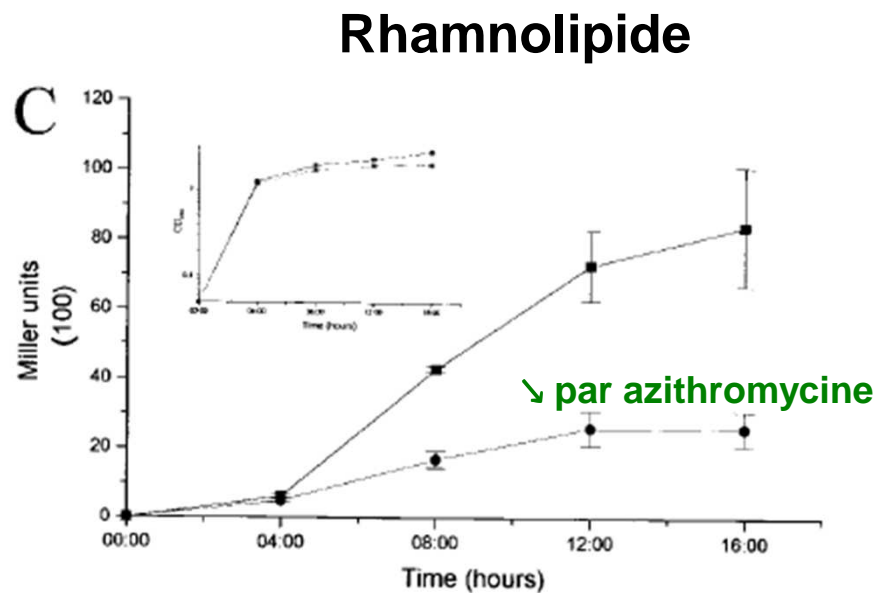
## Inhibiteurs du QS



# Inhibition QS

## Inhibiteur du QS : azithromycine

---

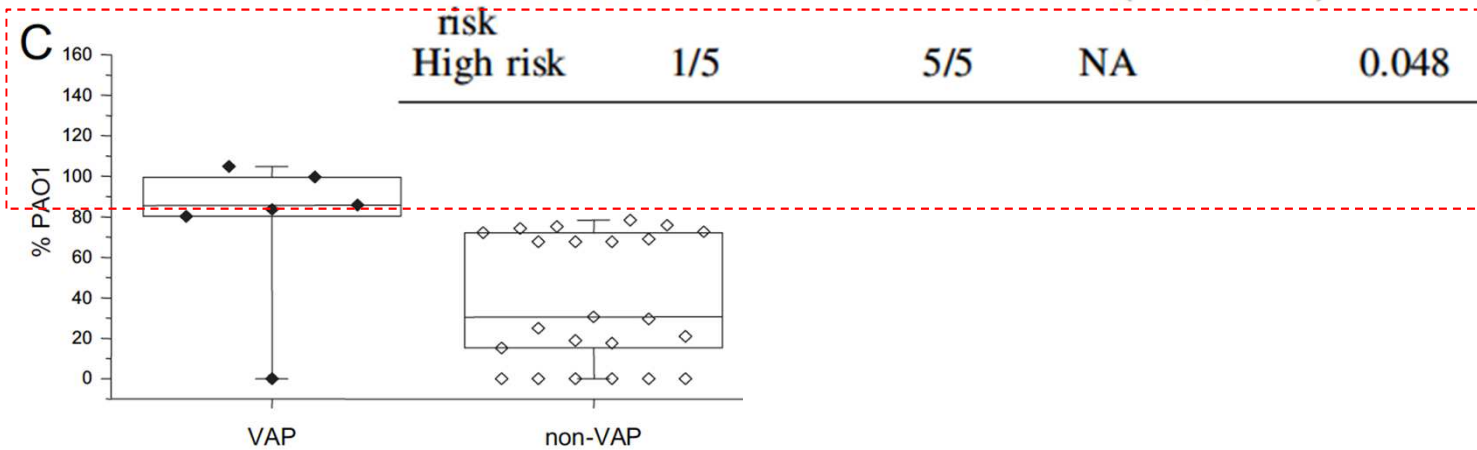


Diminution de la production de facteurs de virulence QS-dependants  
In vitro, sans bactéricidie **sous faible dose d'azithromycine**

# Quorum-sensing

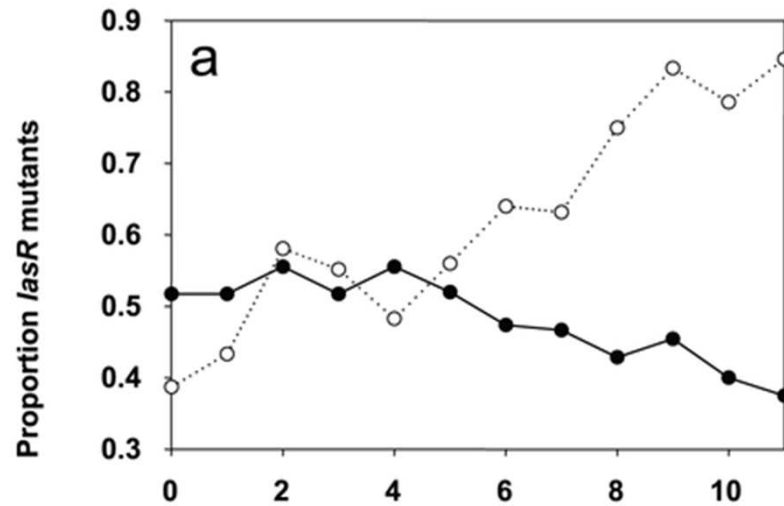
## Inhibiteur du QS : azithromycine

<i>P. aeruginosa</i> VAP	Azithromycin	Placebo	Odds ratio (CI 95 %)	<i>p</i> value <sup>a</sup>
Per-protocol set	2/43	6/42	3.42 (0.65; 18.00)	0.156
According to rhamnolipid-dependent risk				
High and intermediate risk	1/23	5/18	8.46 (0.89; 80.59)	0.070
High risk	1/5	5/5	NA	0.048

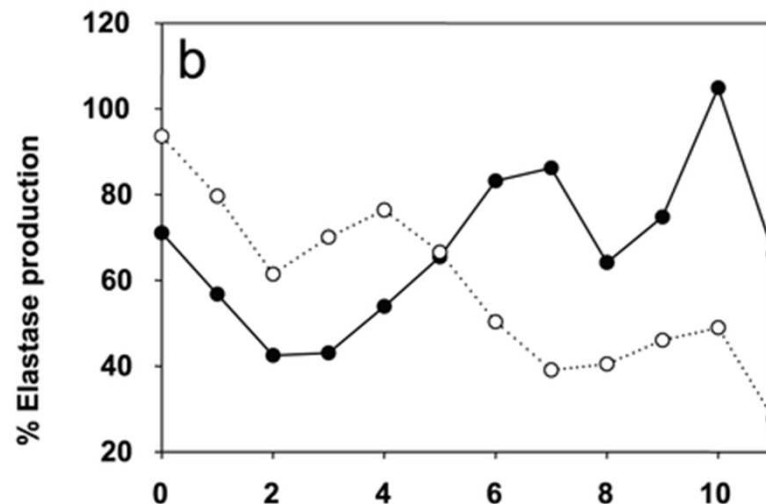


# Inhibition QS

## Limites des inhibiteurs du Quorum-Sensing



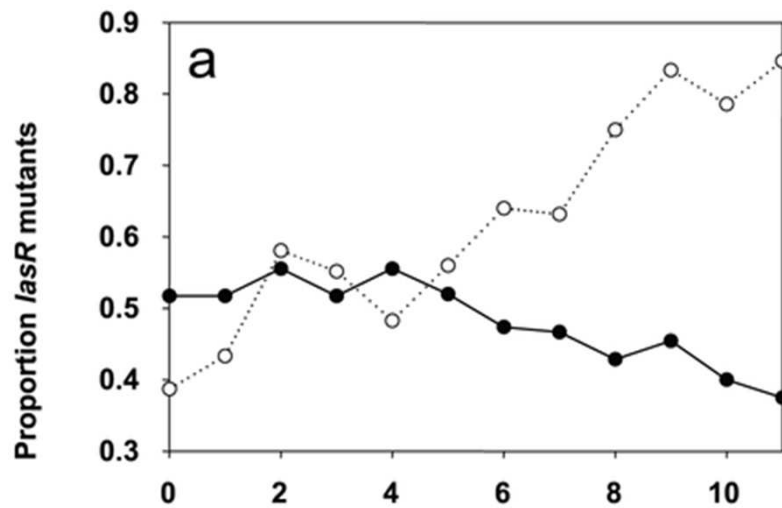
- **SANS azithromycine :**
  - expansion des mutants *lasR*
  - « *tricheurs* » QS-déficients : profitent des ressources de la communauté QS,
  - sans dépenses liées à la communication QS



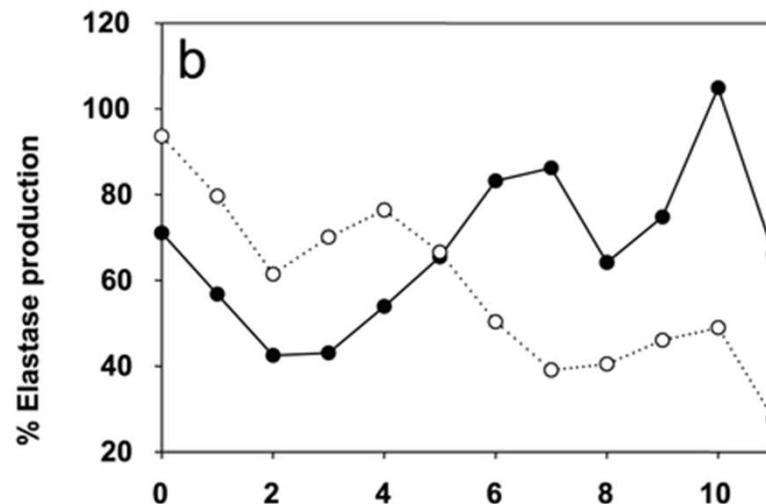
- **en parallèle :**
  - diminution de la virulence QS
  - elastase

# Inhibition QS

## Limites des inhibiteurs du Quorum-Sensing



- **AVEC azithromycine :**
  - diminution mutants *lasR* qui perdent leur avantage



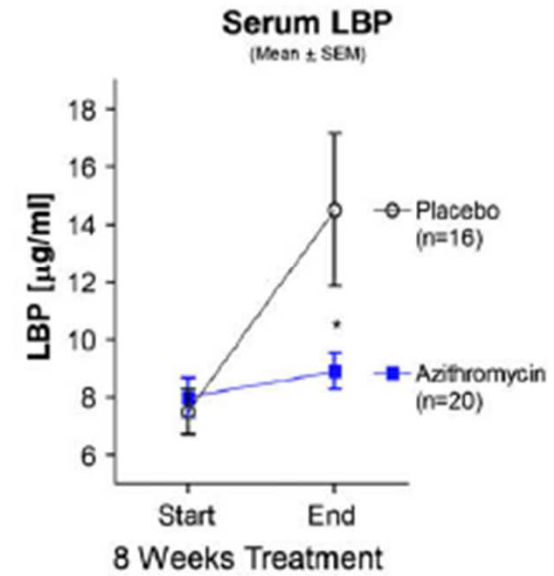
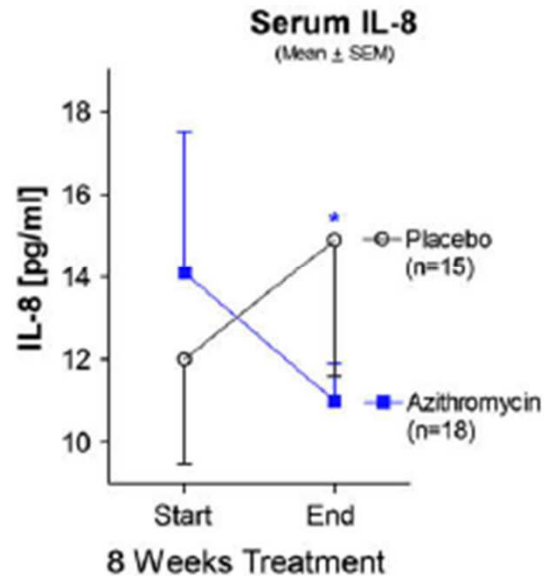
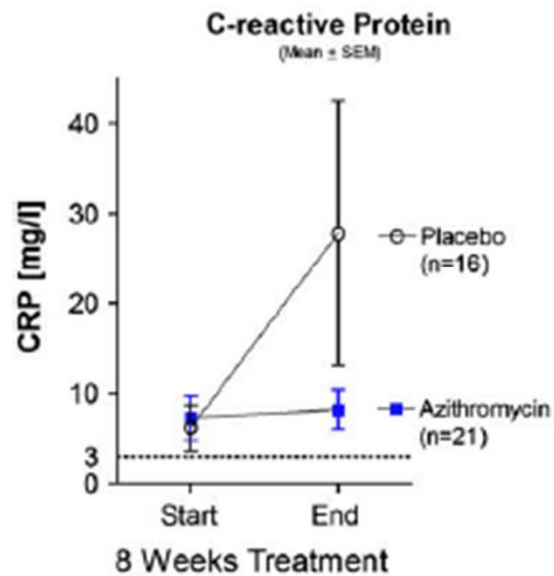
- **en parallèle :**
  - augmentation de la virulence QS!
  - sélection de mutants QS+ qui ont un autre avantage que le QS : une plus grande virulence!



# Inhibition QS

## Azithromycine et infection chronique

38 patients mucoviscidose (21 AZM/17 placebo)  
Etude randomisée contrôlée Azithromycine vs. placebo  
1 fois par semaine pdt 8 semaines



# Inhibition QS

## Azithromycine et infection chronique

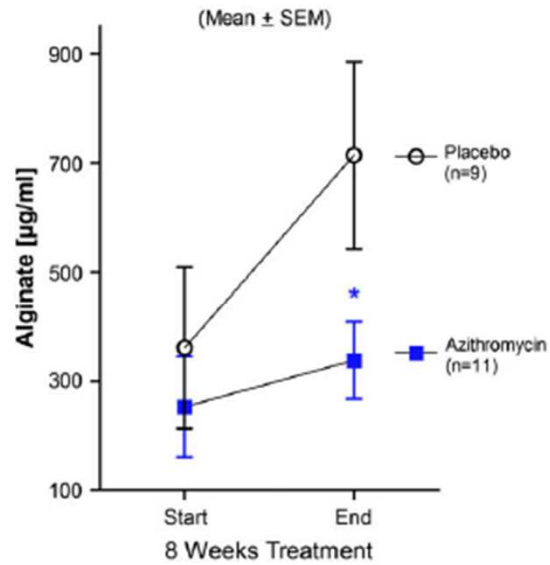


Figure 3 Alginat in sputum.

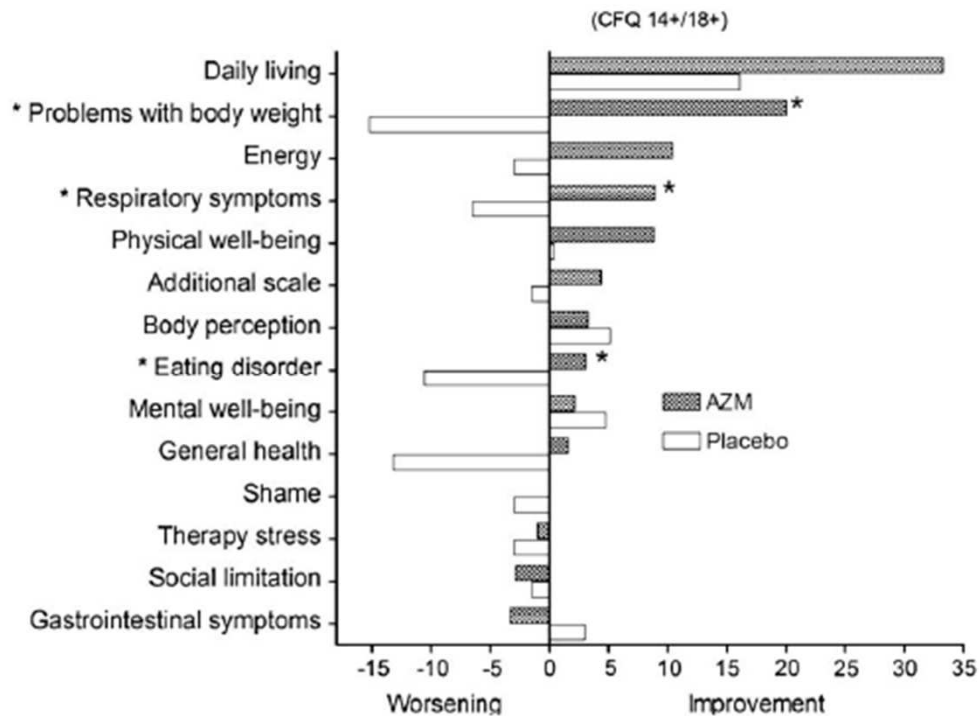


Figure 4 Mean changes in quality of life.

# Cibles et voies thérapeutiques

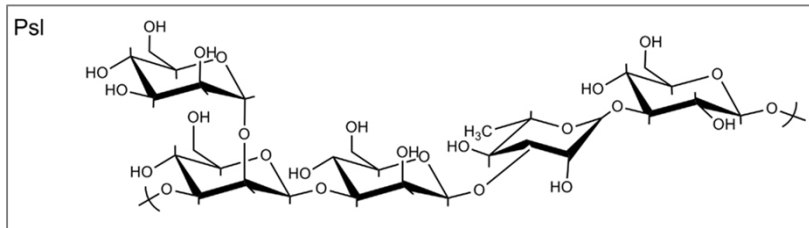
---

*Cibles multiples ?*

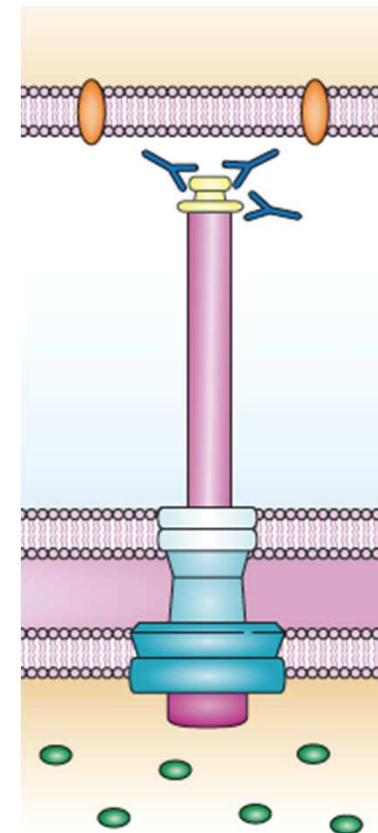
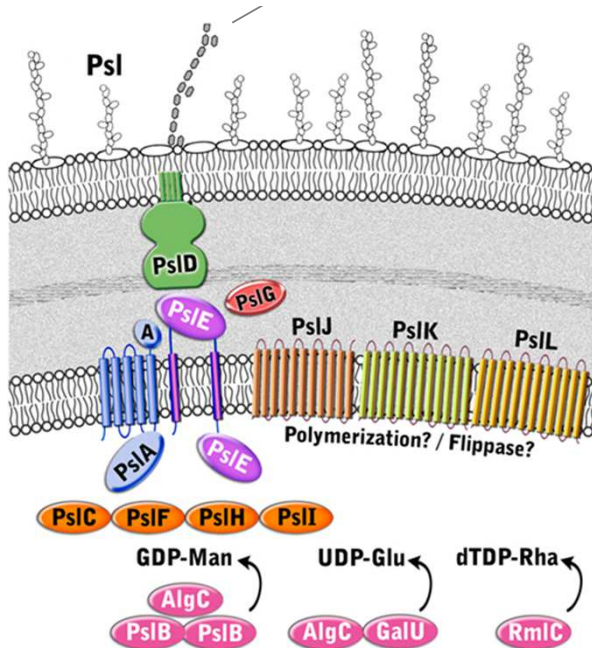
# Cibles multiples

## Anti-biofilm ET Anti-SST3

anticorps bi-spezifique anti-Psl/anti-PcrV



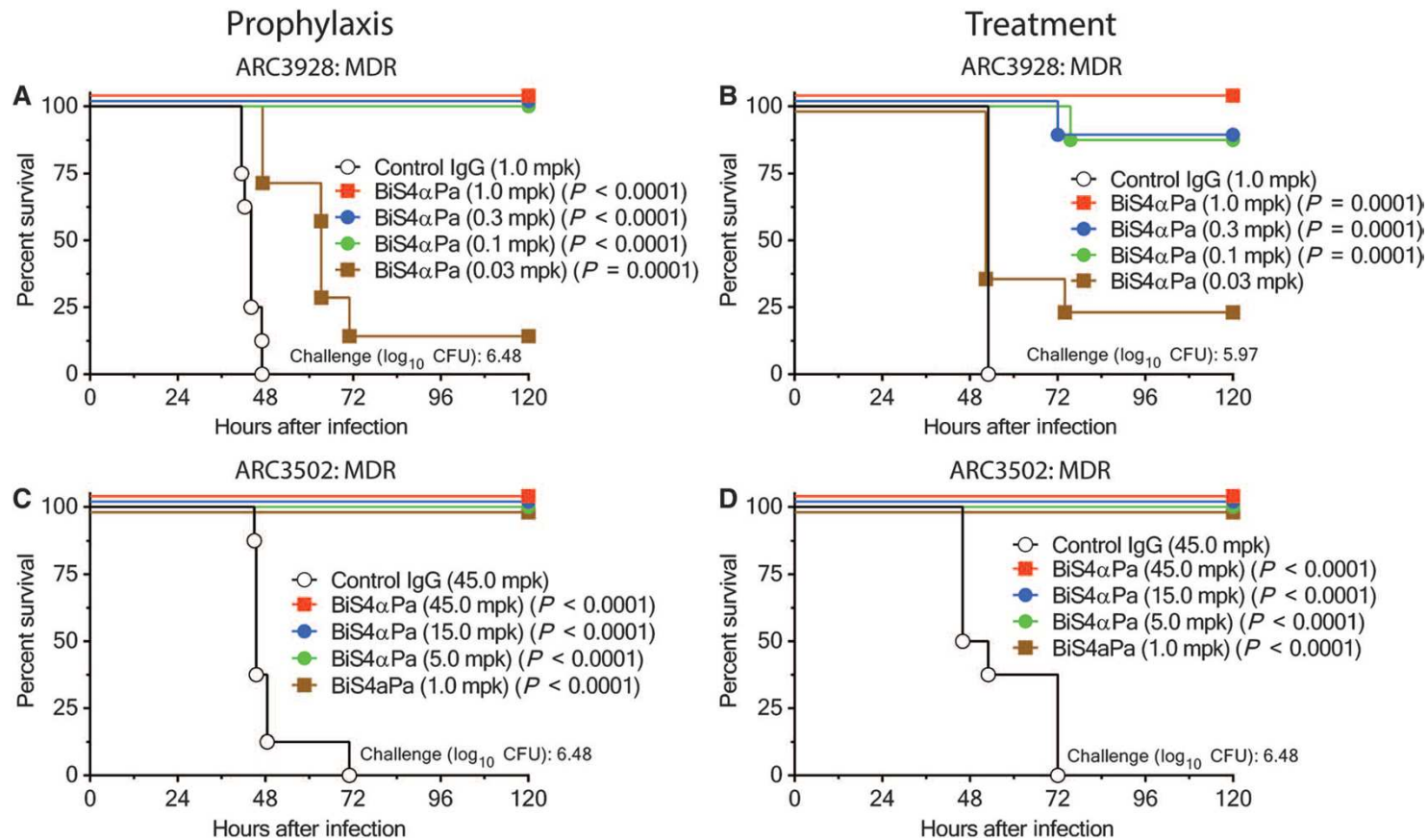
exopolysaccharide du biofilm Psl



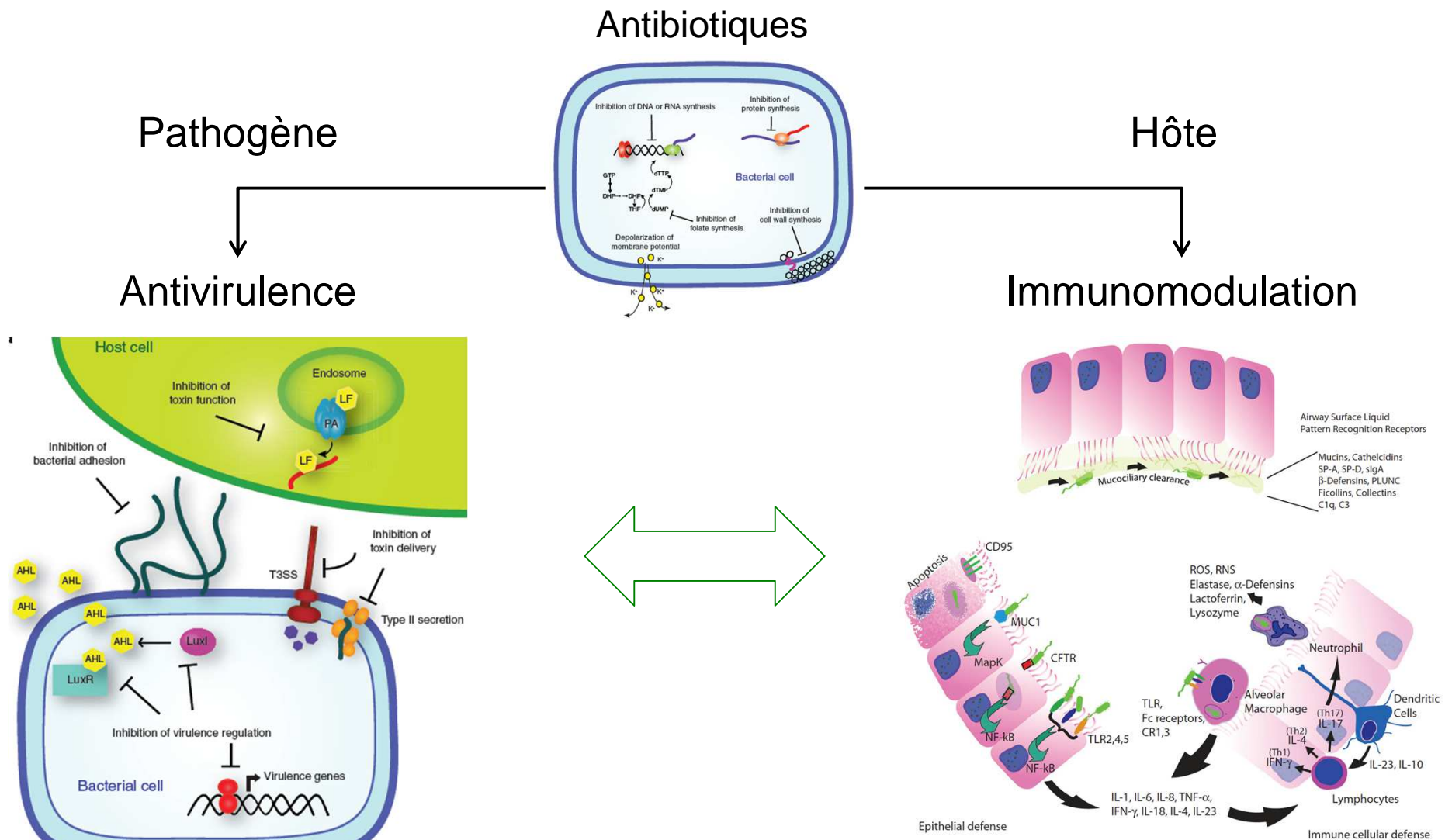
# Cibles multiples

## Anti-biofilm ET Anti-SST3

anticorps bi-spezifique anti-Psl/anti-PcrV



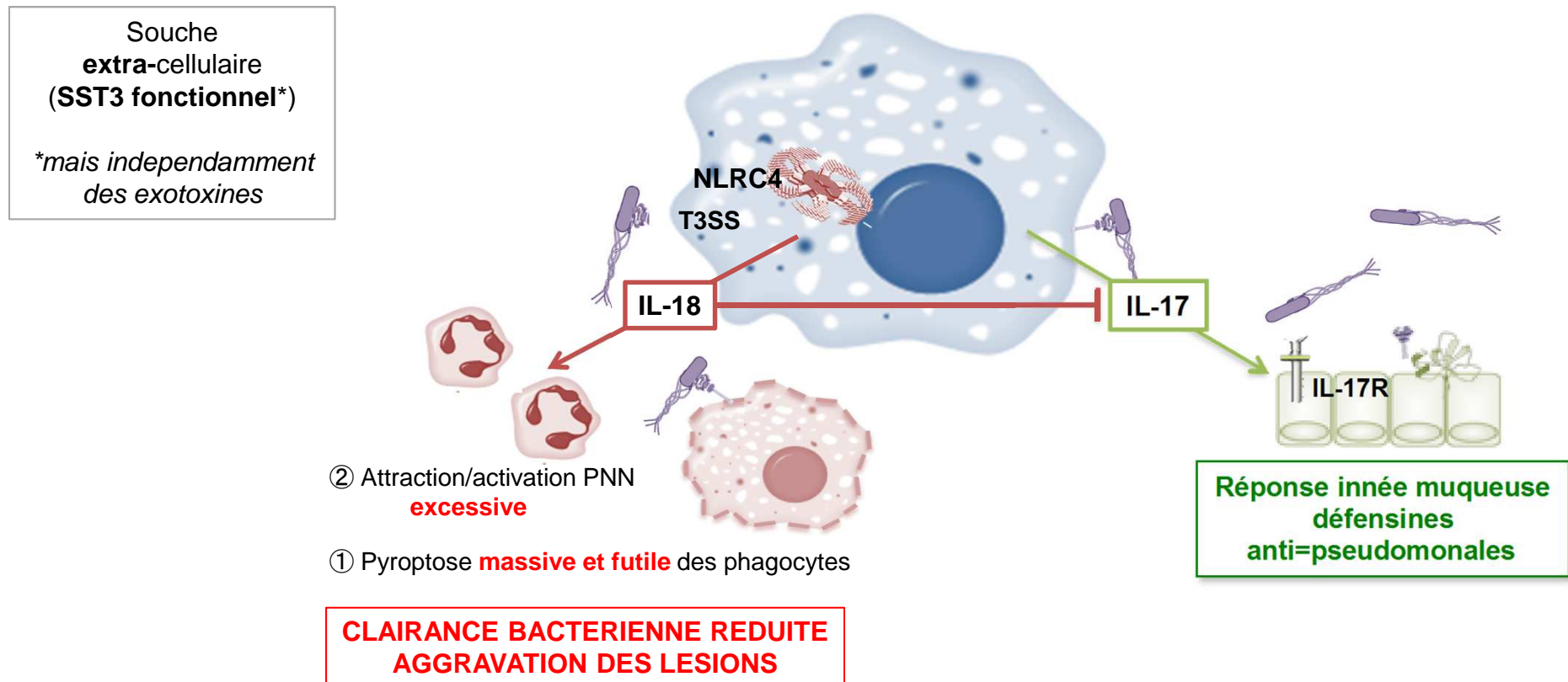
# Lien antivirulence / immunomodulation



# Immunomodulation

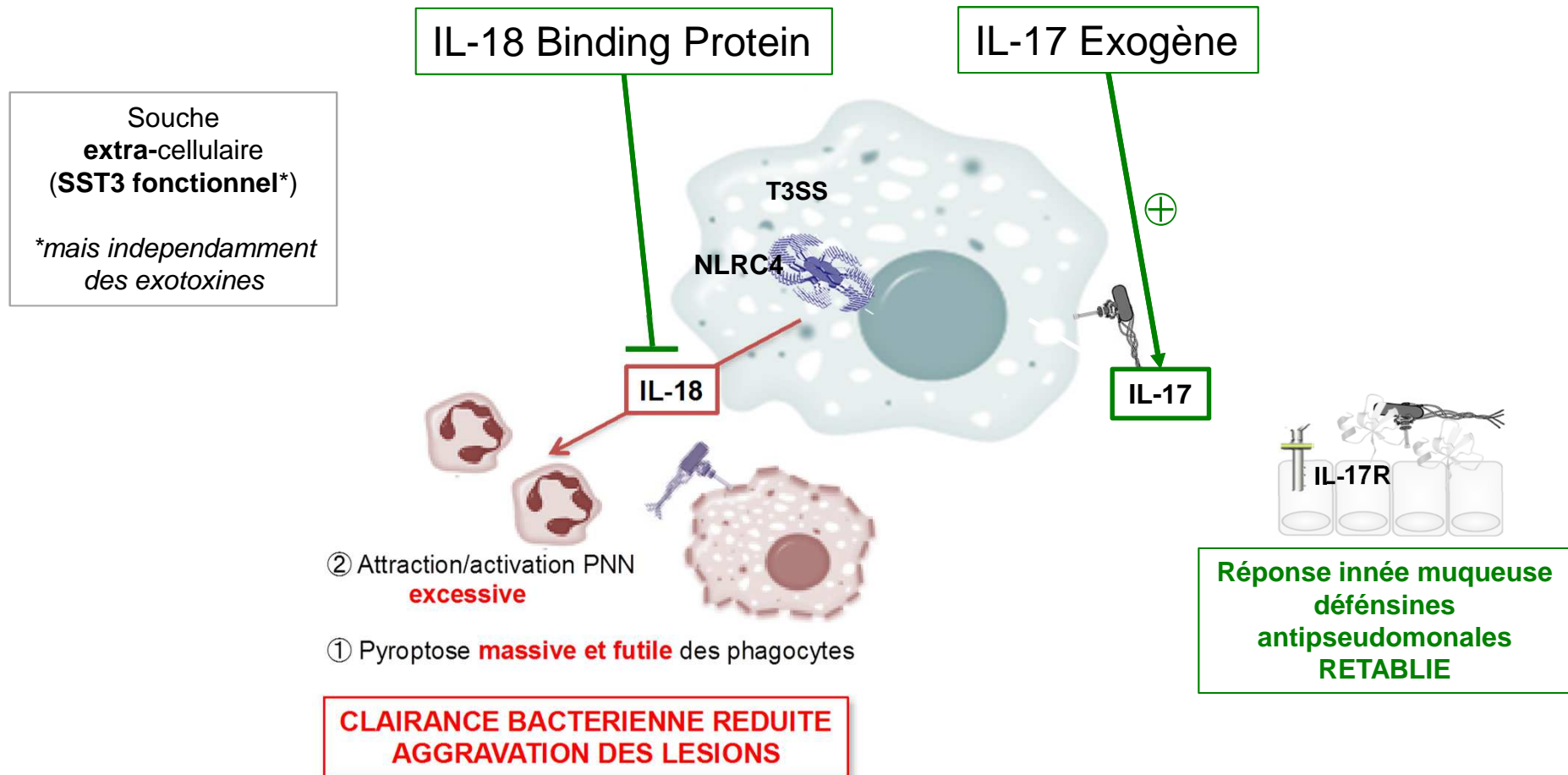
## Le SST3 exploite la réponse innée NLRC4 l'inflammasome

...et réprime une autre réponse innée bénéfique



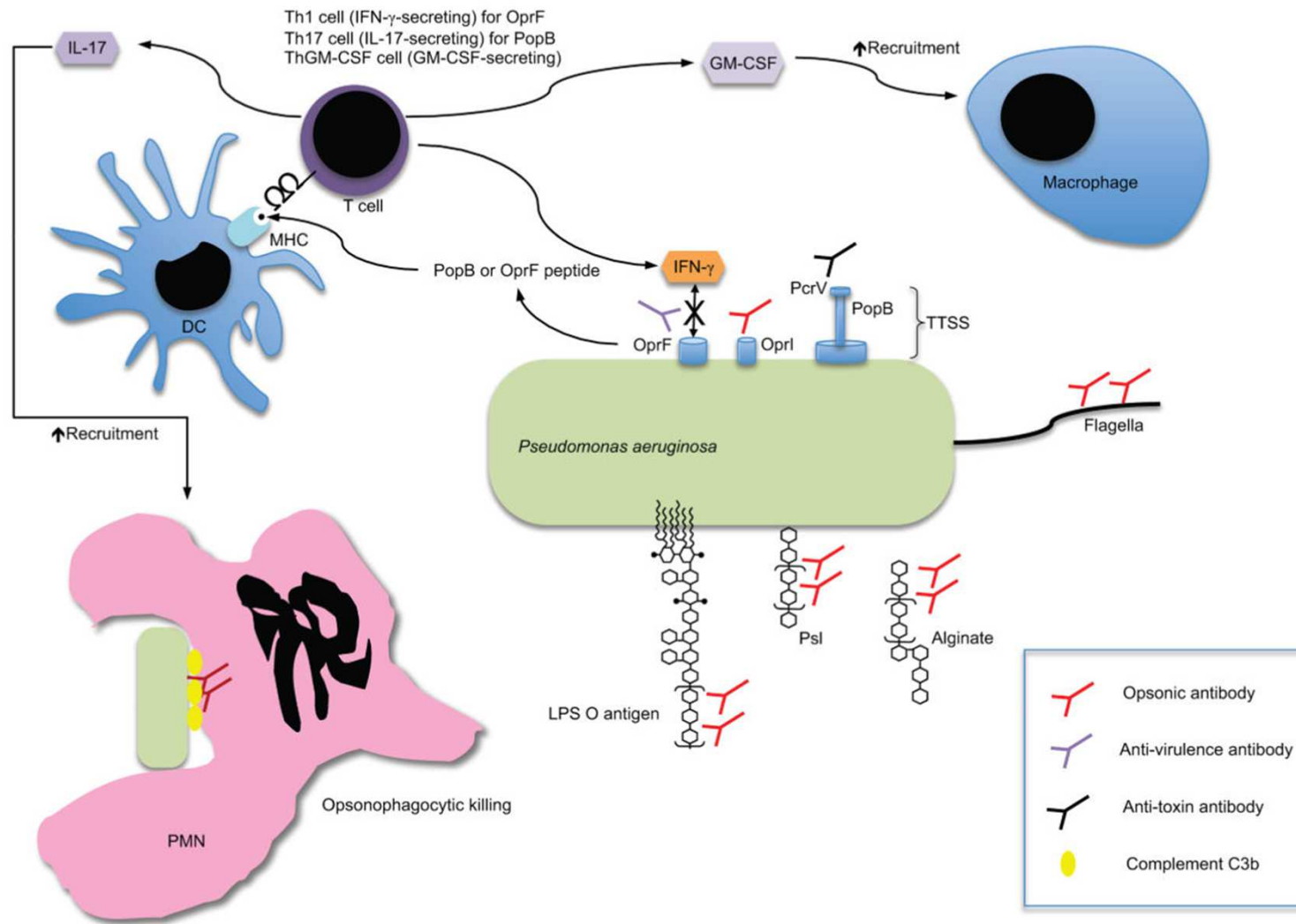
# Immunomodulation

## Immunomodulation thérapeutique ?





# Antivirulence/immunomodulation



# Conclusions

---

- Voies thérapeutiques à explorer
- Intérêt de choisir des cibles également impliquées dans l'immunomodulation
- Intérêt d'avoir des cibles multiples
- Perspectives à explorer en utilisation adjuvante des antibiotiques sur des souches multirésistantes

*merci !*

---

Equipe EA 7366 : Recherche Translationnelle - Relations Hôte-Pathogènes

Benoit Guery



Eric Kipnis  
Karine Faure  
Rodrigue Dessein



Emmanuel Faure  
Perrine Bortolotti  
Marion Thiepaut  
Teddy Grandjean

