

The peptide SET-M33 as novel agent to neutralize and remove LPS in patients with sepsis

Alessandro Pini

pinia@unisi.it

SETLANCE

via Fiorentina 1, 53100 Siena, Italy
www.setlance.com

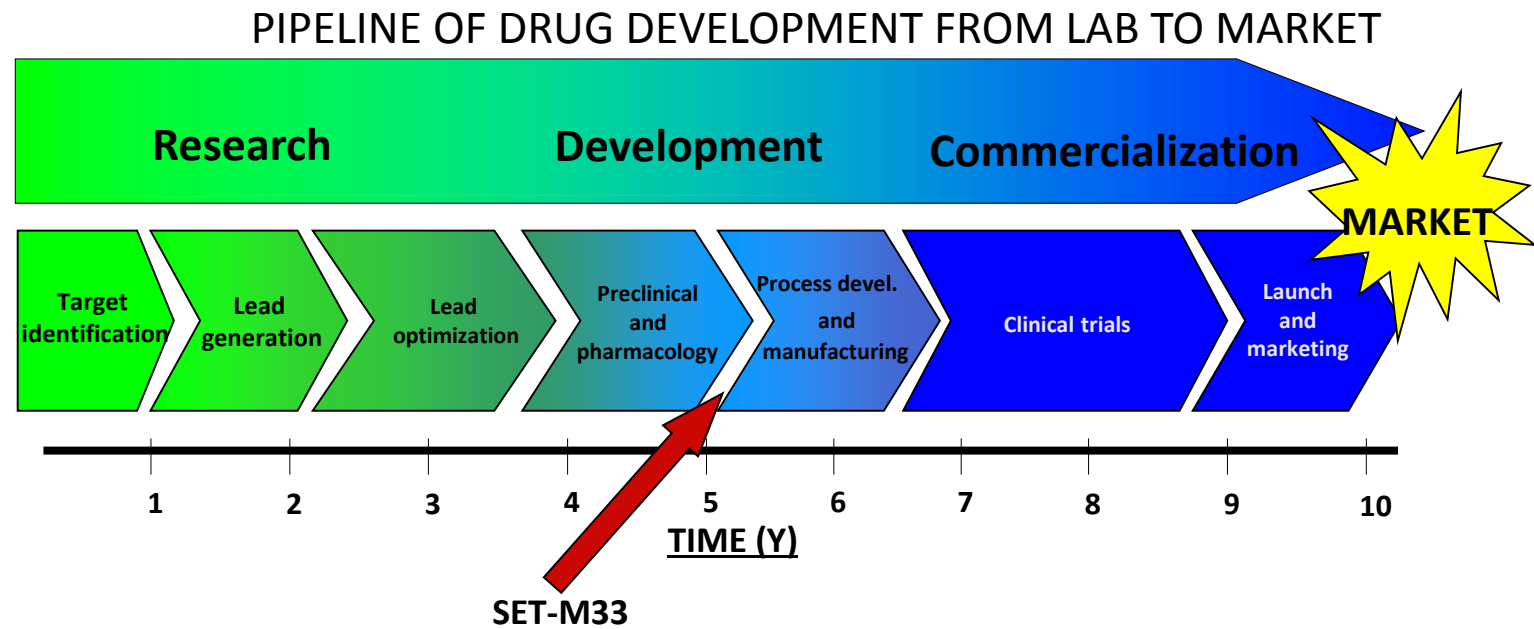
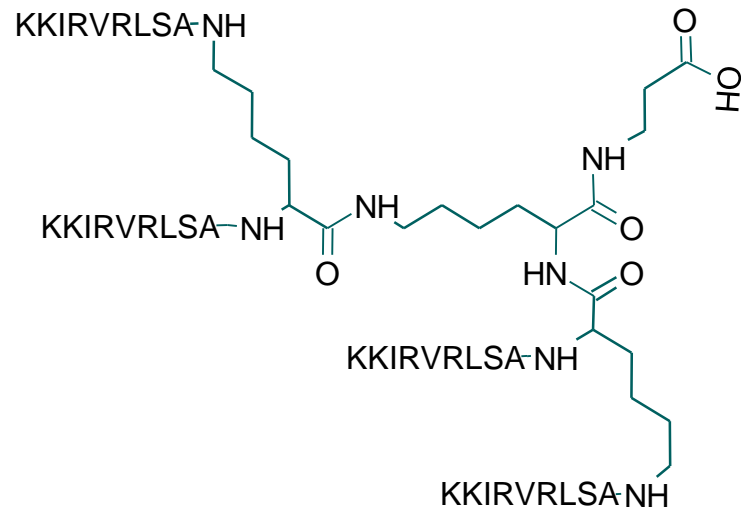


University of Siena
via A. Moro 2, 53100 Siena, Italy

Transparency Declaration: This project received fundings from the European Union's FP7, under grant agreement 604434 and from Fondazione Fibrosi Cistica (FFC#17/2016).
Alessandro Pini is Associate Professor of Biochemistry at the University of Siena and founder and partner of Setlance srl



The antimicrobial peptide SET-M33



Pini et al, 2005, Antimicrob Agents Chemother; Pini et al, J Pept Sci; Pini et al, 2010, FASEB J; Pini et al, 2012 AminoAcids; Falciani et al, 2012 Plos One; Falciani et al, 2014 Aminoacids; Brunetti et al, 2016, Sci Reports; Brunetti et al, 2016, J Biol Chem; Pollini et al, 2017, J Pept Sci;



The antimicrobial peptide SET-M33



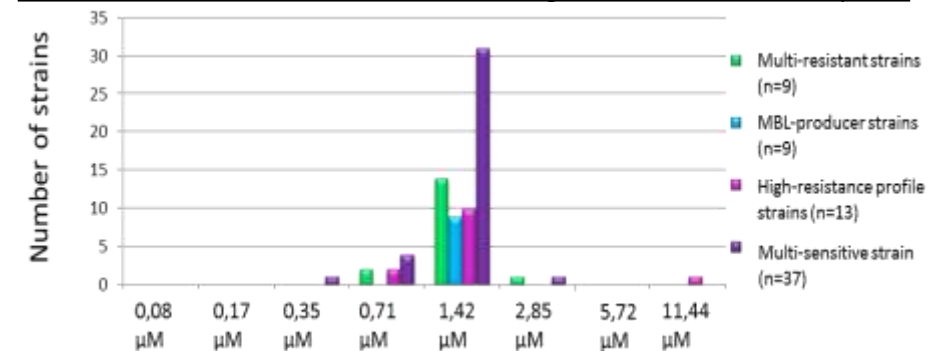
antimicrobial activity *in vitro*

Species and strains	Resistance ^a	MIC (μM)	
		M33	Polymyxin B
<i>Pseudomonas aeruginosa</i> ATCC 27853	Reference strain, wild type	1.5	1.5
<i>P. aeruginosa</i> PAO-1	Reference strain, wild type	1.5	1.5
<i>P. aeruginosa</i> VR-143/97	FQ ^r AG ^r ESC ^r NEM ^r (MBL/VIM-1)	1.5	1.5
<i>P. aeruginosa</i> SC-MDr03-06 ^b	FQ ^r AG ^r ESC ^r NEM ^r	3	1.5
<i>P. aeruginosa</i> SC-VMr04-05 ^b	FQ ^r AG ^r ESC ^r NEM ^r	3	1.5
<i>P. aeruginosa</i> SC-DMr05-04 ^b	FQ ^r AG ^r ESC ^r NEM ^r	1.5	1.5
<i>P. aeruginosa</i> SC-BGr12-02 ^b	FQ ^r AG ^r ESC ^r NEM ^r	1.5	1.5
<i>P. aeruginosa</i> EF-OBG6-1 ^b	FQ ^r AG ^r ESC ^r NEM ^r (MBL/IMP-13)	1.5	0.7
<i>P. aeruginosa</i> SC-MDm03-02 ^{b,c}	FQ ^r AG ^r ESC ^r NEM ^r	3	1.5
<i>P. aeruginosa</i> SC-GMm03-05 ^{b,c}	FQ ^r AG ^r ESC ^r NEM ^r	1.5	1.5
<i>P. aeruginosa</i> SC-CNm03-07 ^{b,c}	FQ ^r AG ^r ESC ^r NEM ^r	0.3	0.7
<i>Klebsiella pneumoniae</i> ATCC 13833	Reference strain, wild type	1.5	0.7
<i>K. pneumoniae</i> 7086042	FQ ^r AG ^r ESC ^r NEM ^r (MBL/VIM-1)	3	1.5
<i>K. pneumoniae</i> C8-27	FQ ^r AG ^r ESC ^r ETP ^r (ESBL/CTX-M-15)	1.5	0.7
<i>K. pneumoniae</i> FIPP-1	FQ ^r AG ^r ESC ^r NEM ^r (MBL/KPC-3)	3	1.5
<i>Escherichia coli</i> ATCC 25922	Reference strain, wild type	1.5	0.7
<i>E. coli</i> W03BG0025	FQ ^r AG ^r ESC ^r (ESBL/CTX-M-15)	0.7	0.7
<i>Enterobacter aerogenes</i> W03BG0067	AG ^r ESC ^r (ESBL/SHV-5)	1.5	0.7
<i>Enterobacter cloacae</i> W03AN0041	ESC ^r (ESBL/SHV-12)	1.5	0.7
<i>Acinetobacter baumannii</i> RUH 134	Reference strain, European clone II	1.5	1.5
<i>A. baumannii</i> RUH 875	Reference strain, European clone I	3	1.5
<i>A. baumannii</i> MR157	FQ ^r AG ^r ESC ^r NEM ^r (OXA/OXA-58)	3	1.5
<i>Staphylococcus aureus</i> ATCC 29213	Reference strain, PEN ^r	6	96
<i>S. aureus</i> 3851	MR VAN ^r	6	96

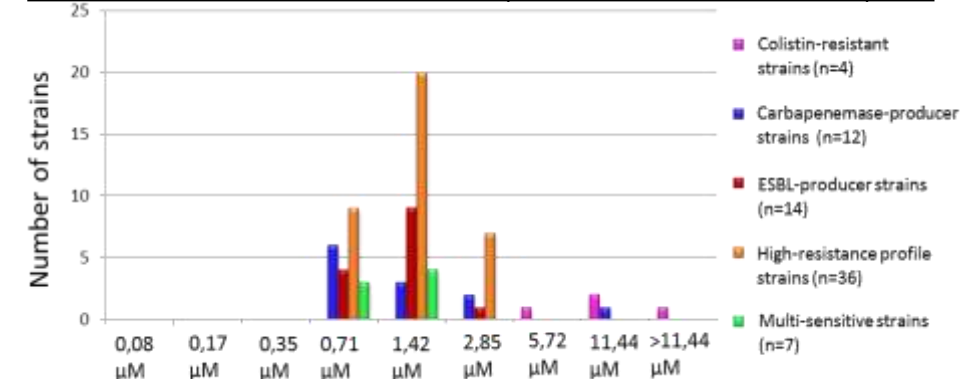
Pini A et al, 2010, FASEB J

Bacterial species	MIC 50	MIC 90
<i>P. aeruginosa</i> , 76 strains	1,4 μM	1,4 μM
<i>K. pneumoniae</i> , 73 strains	1,4 μM	2,8 μM

MIC value distribution with correlation to *P. aeruginosa* bacterial resistance profile



MIC value distribution with correlation to *K. pneumoniae* bacterial resistance profile



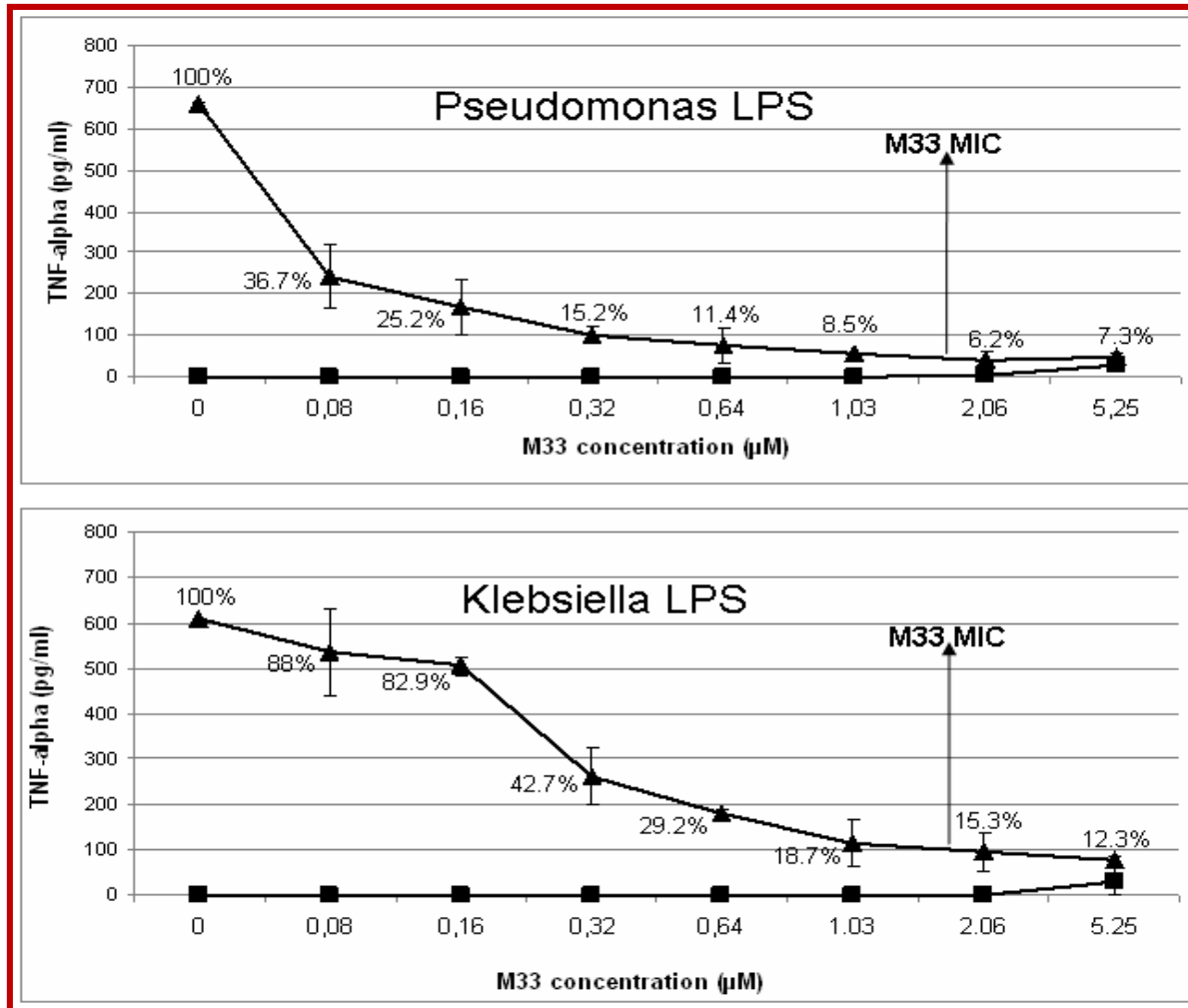
Brunetti J et al, 2016, Sci Rep



The antimicrobial peptide SET-M33



LPS neutralization



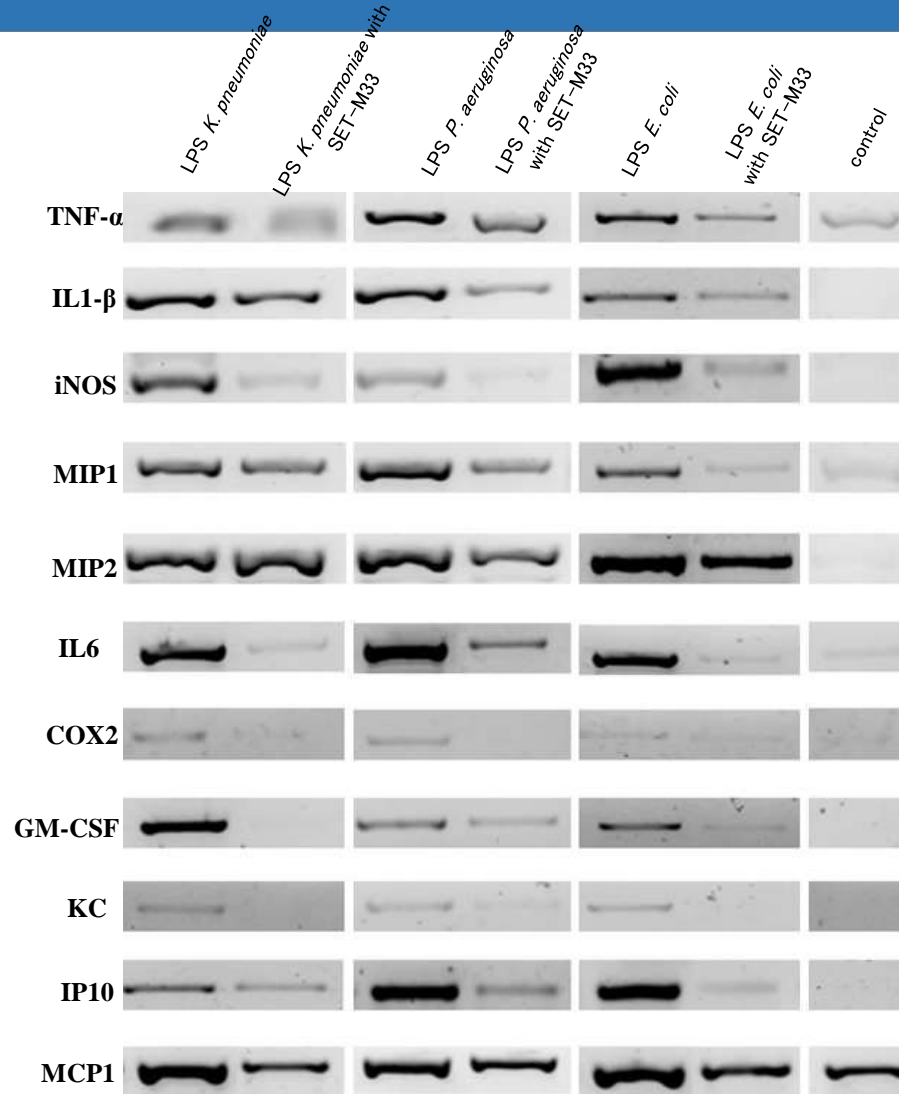
Inhibition of TNF- α release by LPS neutralization. Raw 264.7 (mouse leukaemic monocyte macrophage cells) were incubated with LPS from *P. aeruginosa* and *Klebsiella pneumoniae* and SET-M33. Triangles indicates incubation with LPS and different concentrations of M33. Squares indicates incubation with M33 only.



The antimicrobial peptide SET-M33



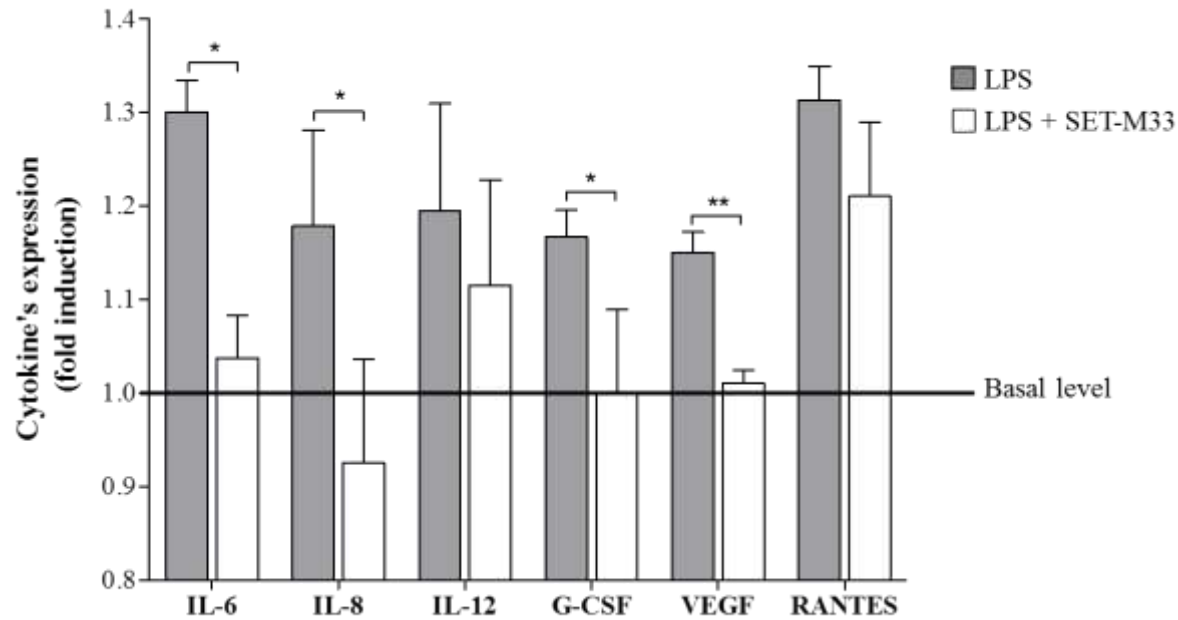
LPS neutralization



	Gene expression percentage		
	LPS <i>K. pneumoniae</i> with SET-M33	LPS <i>P. aeruginosa</i> with SET-M33	LPS <i>E. coli</i> with SET-M33
TNF-α	41.7	4.3	14.6
IL1-β	62.5	22.4	41.2
iNOS	16.7	21.6	18.5
MIP1	60	18.6	15.7
MIP2	100	51.5	68.6
IL-6	3.7	6.3	3.7
COX2	16	18	44.4
GM-CSF	3.5	51.1	18.5
KC	0	44.5	0
IP10	42.9	4.3	0
MCP1	0	24.3	4.9

LPS neutralization

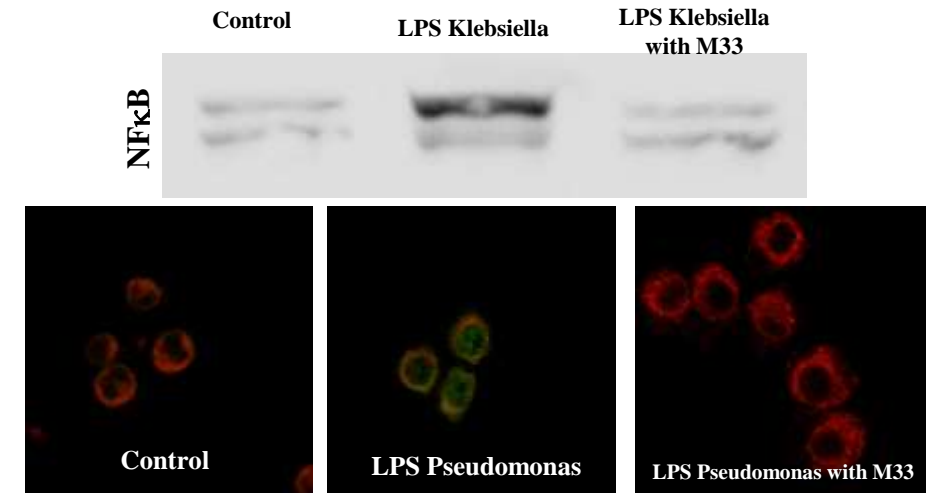
Cytokines' inhibition in CF cells



IB3-1 cells incubated with LPS or with LPS and M33

Bio-plex analysis of cytokines expressed in IB3-1 cells (CF patient) induced with LPS from *P. aeruginosa* and treated with SET-M33. Data is mean \pm SD. Protein expression is reported in relative expression units (fold induction) on the y-axis * $p < 0.05$, ** $p < 0.01$.

NFkB Protein inhibition in macrophages



RAW264.7 cells incubated with LPS or with LPS and M33

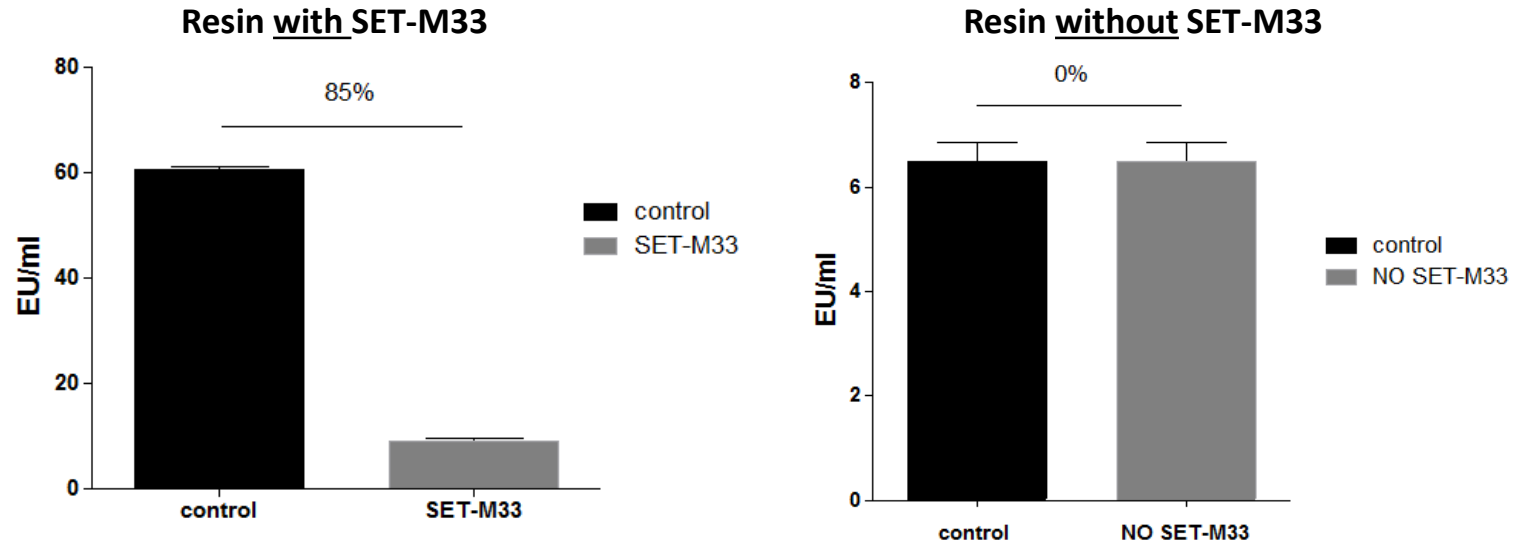
Control = cells not incubated. LPS Pseudomonas = cells stimulated with LPS and producing **NFkB** (green signal). LPS Pseudomonas with M33 = cells incubated with LPS and M33 where the green signal is disappeared



The antimicrobial peptide SET-M33



LPS removal from biological fluids



E. coli LPS removal from human serum incubated with the **resin conjugated with SET-M33**. Y axis indicates amount of endotoxin units per ml. Control histogram is the serum containing LPS before passage into the column. SET-M33 histogram is the same serum after passage into the column. SD is indicated as bars above the histograms

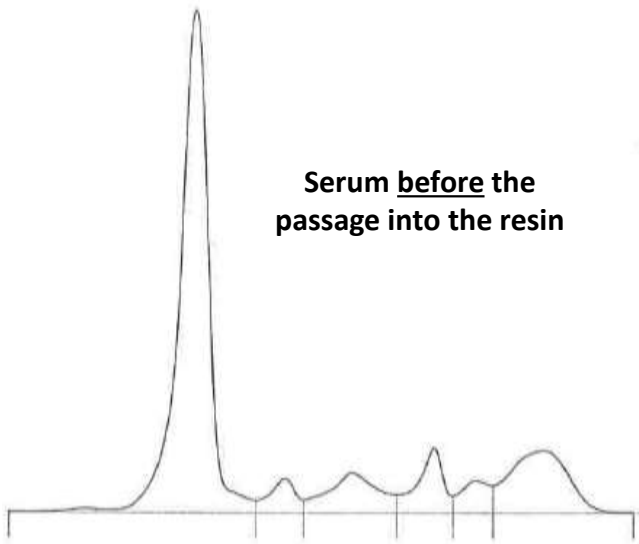


The antimicrobial peptide SET-M33

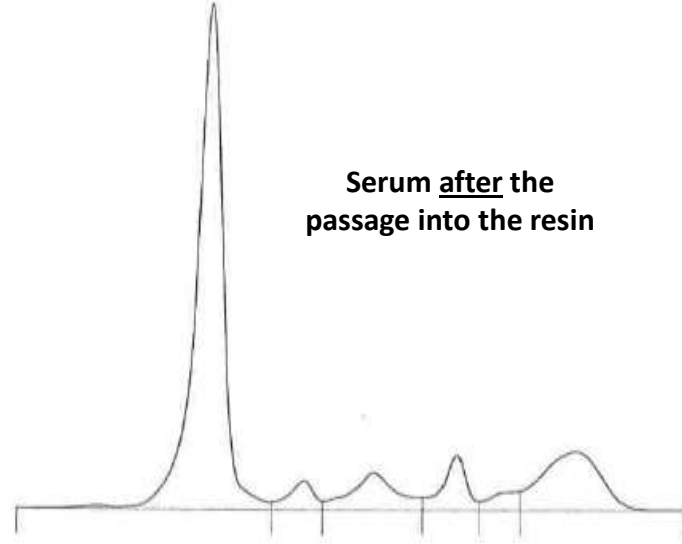


LPS removal from biological fluids

Serum before the passage into the resin



Serum after the passage into the resin



Protein electrophoresis profiles of a serum sample before its passage into the resin (LEFT), and the same serum sample after having been in contact with a resin.

Fractions	%
Albumin	61,4
Alpha 1	3,8
Alpha 2	8,6
Beta 1	7,0
Beta 2	3,9
Gamma	15,3

Fractions	%	Ref. %
Albumin	61,8	55,8-66,1
Alpha 1	3,9	2,9-4,9
Alpha 2	9,2	7,1-11,8
Beta 1	6,4	4,7-7,2
Beta 2	3,5	3,2-6,5
Gamma	15,2	11,1-18,8



THANKS



University of Siena



Jlenia Brunetti
Leila Quercini
Chiara Ambrosini
Stefano Bindi
Silvia Scali
Chiara Falciani
Bracci Luisa

University of Florence



Simona Pollini
Gian Maria Rossolini



www.setlance.com

Giulia Roscia
Davide Donati

BEAM Alliance Members

33 SME in 10 EU Member States

<http://beam-alliance.eu/>