Models *"in vitro"* i *"ex vivo"* per testar activitat antituberculosa.

BRN Seminars. Scientific workshops to faster collaborative research.
Barcelona, 18 de Gener 2018.

Griselda Tudó/ Julià González
Servei de Microbiologia-CDB.
Hospital Clínic de Barcelona-ISGlobal.
Departament de Fonaments Clínics.
Facultat de Medicina i Ciències de la Salut.
Universitat de Barcelona.

Per què

Bases del tractament combinat en tuberculosi: Administració de diferents fàrmacs que actuen sobre diferents dianes i *estatus* metabòlics existents en una lesió tuberculosa. S'evita l'aparició i selecció de resistències que poden inutilitzar per sempre aquests fàrmacs.

Table 2 The clinical development phases

Phase I trials	Initial studies to determine the metabolism and pharmacologic actions of drugs in humans, the side effects associated with increasing doses, and to gain early evidence of effectiveness; may include healthy participants or patients.
Phase II trials	Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks. This phase can also be used to establish dose ranges and dose–response relationships. Phase IIa addresses dose and dose–response with limited numbers of participants (typically 30–50), whereas phase IIb engages risks and efficacy with larger numbers of participants (typically 200–500).
Phase III trials	Expanded controlled and uncontrolled trials conducted after preliminary evidence suggesting effectiveness of the drug has been obtained, that are intended to gather additional information to evaluate the overall benefit–risk relationship of the drug and provide adequate basis for physician labeling based on established short and long-term safety and efficacy of the drug.
Phase IV trials	Postmarketing studies to delineate additional information, including the drug's risks, benefits, and optimal use in populations.

Source: http://clinicaltrials.gov/ct2/info/ from ClinicalTrials.gov 2009.

Lienhdart *et al*., 2010

20-30 anys per desenvolupar noves pautes de 3 o 4 fàrmacs

Per què

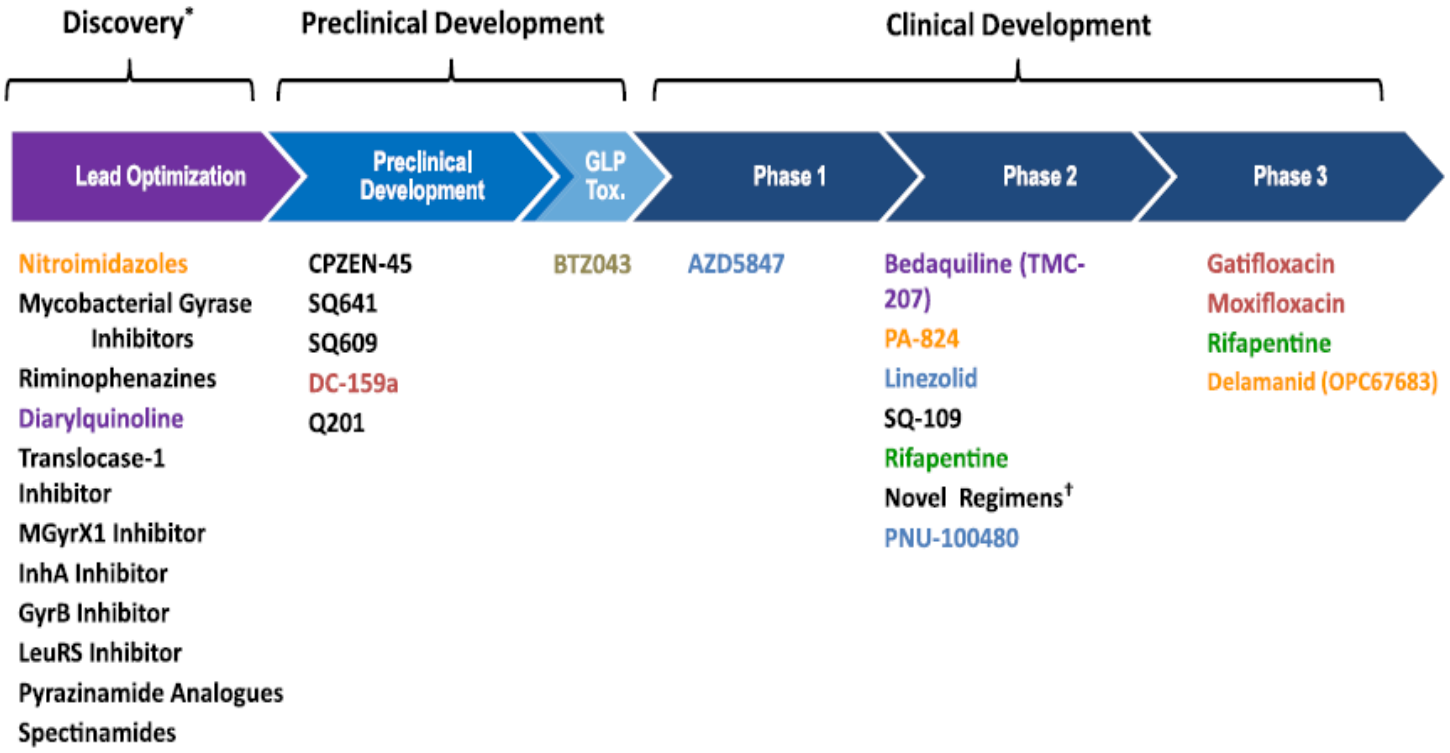


Figure 1. Global tuberculosis drug pipeline. Chemical classes are shown in various colors: fluoroquinolone (*red*), rifamycin (*green*), oxazolidinone (*blue*), nitroimidazole (*yellow*), diarylquinoline (*purple*), enzothiazinone (*tan*). *Ongoing projects without a lead compound series can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php. †Combination regimens: first clinical trial (NC001) of a novel tuberculosis drug regimen testing the 3-drug combination of PA-824, moxifloxacin, and pyrazinamide was initiated in November 2010. Abbreviation: GLP Tox, Good Laboratory Practice Toxicology Study.
Source: Working Group on New Drugs, Stop TB Partnership.

Lienhardt *et al*., 2012

Per què

Model de treball proposat per l'estudi de noves teràpies combinades.

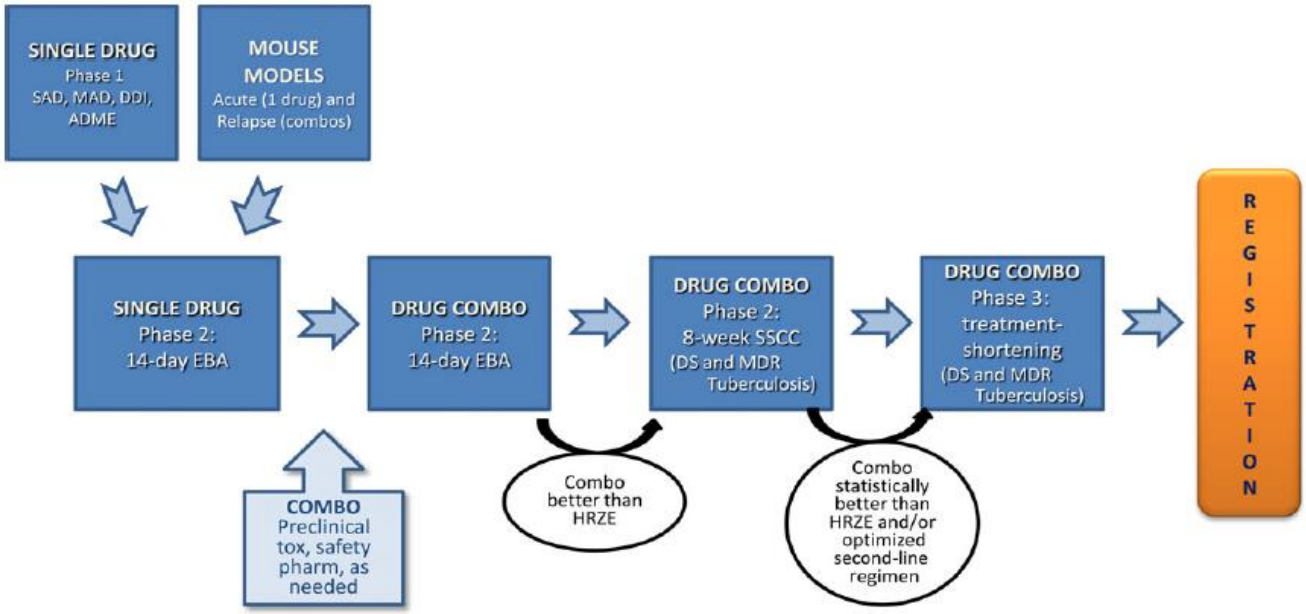


Figure 2. New model for development of drug combination therapy. Abbreviations: ADME, absorption, distribution, metabolism, and excretion; DDI, drug-drug interaction; DS, drug-susceptible; EBA, early bactericidal activity; HRZE, isoniazid, rifampicin, pyrazinamide and ethambutol; MAD, multiple ascending dose; MDR, multidrug-resistant; SAD, single ascending dose; SSCC, serial sputum colony count.

Lienhardt *et al.*, 2012

Mètodes d'estudi

- Mètodes *in vitro*
  - Tauler d'escacs
  - Corbes de letalitat

  } Mètodes bàsics

- Mètodes *ex vivo*
  - Línia cel·lular de macròfags: ex: THP-1
- Models animals

Definicions emprades per l'estudi de la interacció entre antimicrobians

Sinergisme: interacció positiva.

L'efecte de la combinació > l'efecte individual.

Antagonisme: interacció negativa.

L'efecte de la combinació < l'efecte individual.

Indiferent (o additiu):

L'efecte de la combinació=Σ dels efectes individuals.

Mètodes *in vitro*: tauler d'escacs

Valora l'**activitat** *in vitro* de diferents antibiòtics per determinar si la combinació és més activa que qualsevol dels antibiòtics sols.

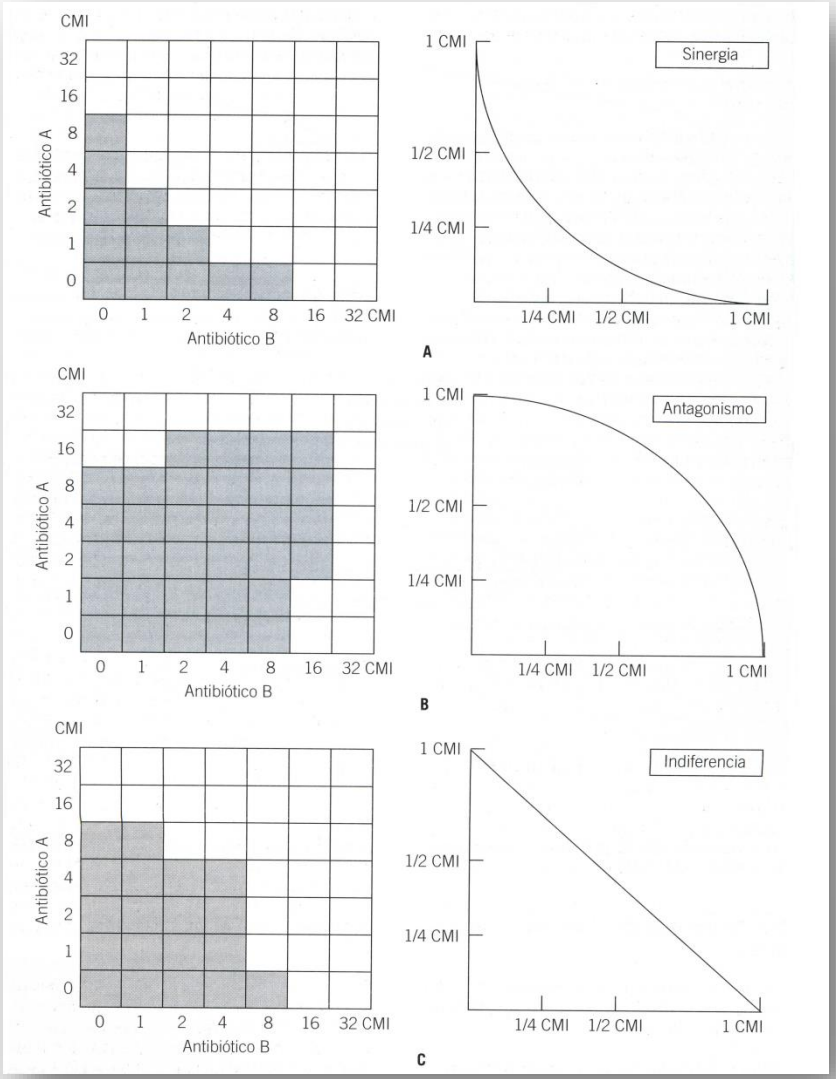
Índex FCI o ΣFIC (Concentració Inhibitòria Fraccionada)

$$IFIC = FIC_A + FIC_B = A/CMI_A + B/CMI_B.$$

A, B són les CMI d'atb en combinació.

CMI_A, CMI_B són les CMI individuals.

Sinergisme FIC< 0.5



Marco *et al*., Modelos experimentales en patología infecciosa. Fundació Dr. Esteve 2000.

Mètodes *in vitro*: tauler d'escacs

Combinacions
INH-RIF-EMB
OFX-RIF-EMB

Table 1
MICs and FICI of the INH, RIF and EMB combination of the isolates studied.

Isolate	Susceptibility pattern	MIC alone (μg/mL)			MIC in combination (μg/mL)			FIC			FICI
		INH	RIF	EMB	INH	RIF	EMB	INH	RIF	EMB	
18098	INH	51.2	0.5	2.5	6.4	0.06	0.31	0.12	0.12	0.12	0.38
14533	INH	0.8	0.5	2.5	0.1	0.06	0.31	0.12	0.12	0.12	0.38
12765	INH	1.6	1	2.5	0.2	0.12	0.31	0.12	0.12	0.12	0.38
63752	INH	0.8	0.5	2.5	0.1	0.06	0.31	0.12	0.12	0.12	0.38
49493	INH	3.2	1	2.5	0.2	0.12	0.31	0.06	0.12	0.12	0.31
39984	INH	51.2	1	2.5	6.4	0.12	0.31	0.12	0.12	0.12	0.38
44016	INH	51.2	1	2.5	6.4	0.12	0.31	0.12	0.12	0.12	0.38
14531	INH	0.8	0.5	2.5	0.1	0.06	0.31	0.12	0.12	0.12	0.38
63734	INH	0.8	0.5	2.5	0.1	0.06	0.31	0.12	0.12	0.12	0.38
63762	INH	0.8	1	2.5	0.1	0.12	0.31	0.12	0.12	0.12	0.38
63753	INH	0.8	1	2.5	0.1	0.12	0.31	0.12	0.12	0.12	0.38
12776	INH	0.8	0.5	2.5	0.1	0.06	0.31	0.12	0.12	0.12	0.38
1S	*	0.02	0.5	2.5	0.01	0.12	0.31	0.5	0.25	0.12	0.88
2S	*	0.02	0.5	2.5	0.01	0.12	0.31	0.5	0.25	0.12	0.88
3S	*	0.02	0.12	2.5	0.01	0.12	0.31	0.5	1	0.12	1.6
4S	*	0.05	0.25	2.5	0.01	0.12	0.31	0.25	0.5	0.12	0.88
5S	*	0.05	0.25	2.5	0.01	0.12	0.31	0.25	0.5	0.12	0.88
6S	*	0.05	0.25	2.5	0.01	0.12	0.31	0.25	0.5	0.12	0.88
7S	*	0.05	0.5	2.5	0.01	0.12	0.31	0.25	0.25	0.12	0.6
8S	*	0.05	0.5	2.5	0.01	0.12	0.31	0.25	0.25	0.12	0.6
9S	*	0.05	0.25	2.5	0.01	0.12	0.31	0.25	0.5	0.12	0.88
10S	*	0.02	0.5	2.5	0.01	0.12	0.31	0.5	0.25	0.12	0.88
H37Rv	*	0.05	0.25	2.5	0.01	0.12	0.31	0.25	0.5	0.12	0.88

MIC: Minimal Inhibitory Concentration; FIC: Fractional Inhibitory Concentration; FICI: Fractional Inhibitory Concentration Index; INH: Isoniazid; RIF: Rifampicin; EMB: Ethambutol. * Drug-susceptible isolates.

Rey-Jurado *et al*. *Tuberculosis (*Edinb). 2012 May;92(3):260-3.

Mètodes *in vitro*: tauler d'escacs

Table 2
MICs and FICI of the OFX, EMB and RIF combination of the isolates studied.

Isolate	Susceptibility pattern	MIC alone (μg/mL)			MIC in combination (μg/mL)			FIC			FICI
		OFX	EMB	RIF	OFX	EMB	RIF	OFX	EMB	RIF	
18098	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
14533	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
12765	INH	1	2.5	1	0.25	0.31	0.12	0.25	0.12	0.12	0.5
63752	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
49493	INH	1	2.5	1	0.25	0.31	0.5	0.25	0.12	0.5	0.87
39984	INH	1	2.5	1	0.25	0.31	0.12	0.25	0.12	0.12	0.5
44016	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
14531	INH	1	2.5	1	0.25	0.31	0.12	0.25	0.12	0.12	0.5
63734	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
63762	INH	1	2.5	0.5	0.25	0.31	0.06	0.25	0.12	0.12	0.5
63753	INH	1	2.5	1	0.25	0.31	0.12	0.25	0.12	0.12	0.5
12776	INH	1	2.5	1	0.25	0.31	0.5	0.25	0.12	0.5	0.87
1S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
2S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
3S	*	0.25	2.5	0.12	0.06	0.31	0.03	0.12	0.12	0.25	0.62
4S	*	0.5	2.5	0.25	0.06	0.31	0.03	0.12	0.12	0.12	0.4
5S	*	0.5	2.5	0.25	0.06	0.31	0.03	0.12	0.12	0.12	0.4
6S	*	0.5	2.5	0.25	0.06	0.31	0.03	0.12	0.25	0.12	0.5
7S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
8S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
9S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
10S	*	0.5	2.5	0.5	0.06	0.31	0.03	0.12	0.12	0.06	0.31
H37Rv	*	0.5	2.5	0.25	0.06	0.31	0.03	0.12	0.12	0.12	0.4

MIC: Minimal Inhibitory Concentration; FIC: Fractional Inhibitory Concentration; FICI: Fractional Inhibitory Concentration Index; OFX: Ofloxacin; EMB: Ethambutol; RIF: Rifampicin; INH: Isoniazid. * Drug-susceptible isolates.

Mètodes *in vitro*: tauler d'escacs

Combinacions
LEV-LNZ-AMK
LEV-LNZ-EMB
LEV-AMK-EMB

Table 2
Effect of three different combinations with levofloxacin (LEV), linezolid (LNZ), amikacin (AMK) and ethambutol (EMB) against drug-susceptible and multidrug-resistant (MDR) *Mycobacterium tuberculosis* isolates.

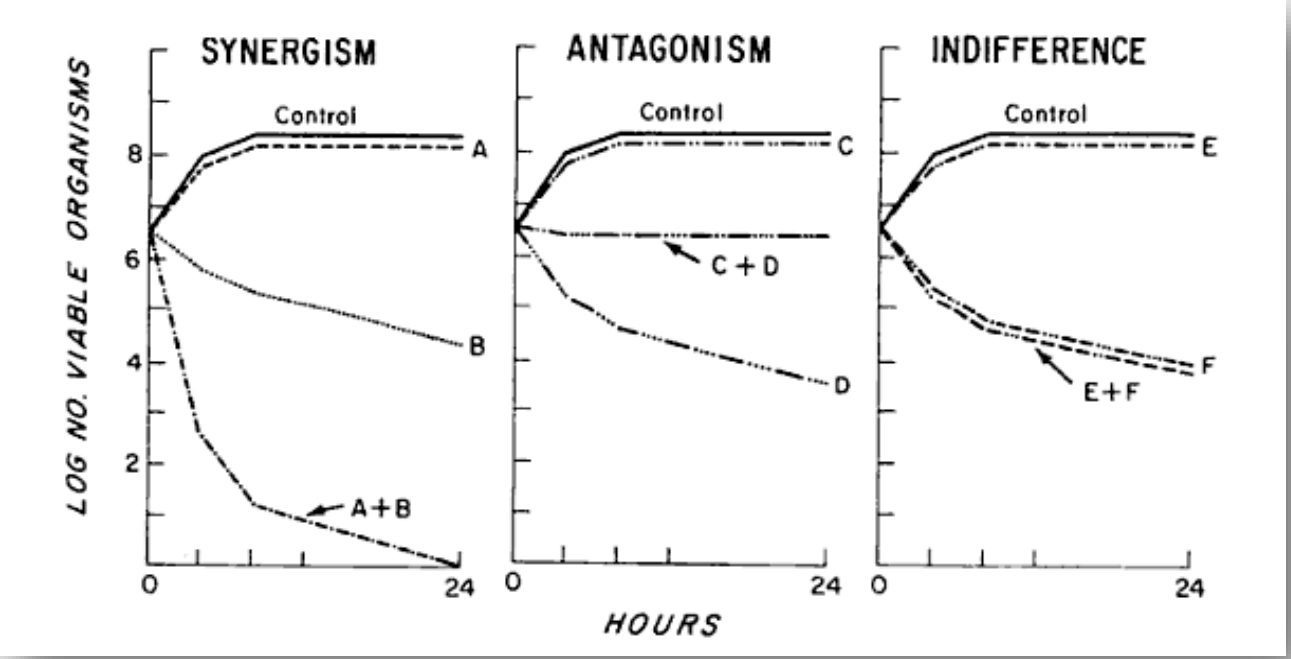
Isolate	Susceptibility pattern	MIC alone (mg/L)				LEV/LNZ/AMK		LEV/LNZ/EMB		LEV/AMK/EMB	
		LEV	LNZ	AMK	EMB	MICs (mg/L)	FICI	MICs (mg/L)	FICI	MICs (mg/L)	FICI
1R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
2R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
3R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
4R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
5R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.25/1.25/1.25	1.5
6R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.25/1.25/1.25	1.5
7R	MDR	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
8R	MDR	0.5	1	2.5	2.5	0.25/0.5/1.25	1.5	0.25/0.5/1.25	1.5	0.5/2.5/2.5	3
9R	MDR	0.5	1	2.5	2.5	0.25/0.5/1.25	1.5	0.25/0.5/1.25	1.5	0.5/2.5/2.5	3
1S	*	0.5	1	2.5	2.5	0.25/0.5/1.25	1.5	0.25/0.5/1.25	1.5	0.5/2.5/2.5	3
2S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
3S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
4S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.5/0.5/2.5	3	0.5/2.5/2.5	3
5S	*	0.5	0.5	2.5	2.5	0.5/0.5/2.5	3	0.5/0.5/2.5	3	0.5/2.5/2.5	3
6S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
7S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.25/0.25/1.25	1.5	0.5/2.5/2.5	3
8S	*	0.5	0.5	2.5	2.5	0.5/0.5/2.5	3	0.5/0.5/2.5	3	0.5/2.5/2.5	3
9S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.5/0.5/2.5	3	0.5/2.5/2.5	3
10S	*	0.5	0.25	2.5	2.5	0.5/0.25/2.5	3	0.25/0.25/1.25	2	0.5/2.5/2.5	3
11S	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.5/0.5/2.5	3	0.5/0.5/2.5	3
H37Rv	*	0.5	0.5	2.5	2.5	0.25/0.25/1.25	1.5	0.5/0.5/2.5	3	0.5/2.5/2.5	3

MIC, minimum inhibitory concentration; FICI, fractional inhibitory concentration index.
* Drug-susceptible isolates.

Rey-Jurado *et al*. *Int J Antimicrob Agents.* 2013 Mar;41(3):278-80.

Mètodes *in vitro*: corbes de letalitat

S'estudia la **dinàmica** de l'activitat dels antibiòtics sols i/o en combinació mitjançant el recompte del nombre de colònies viables durant un període de temps determinat (hores o dies).

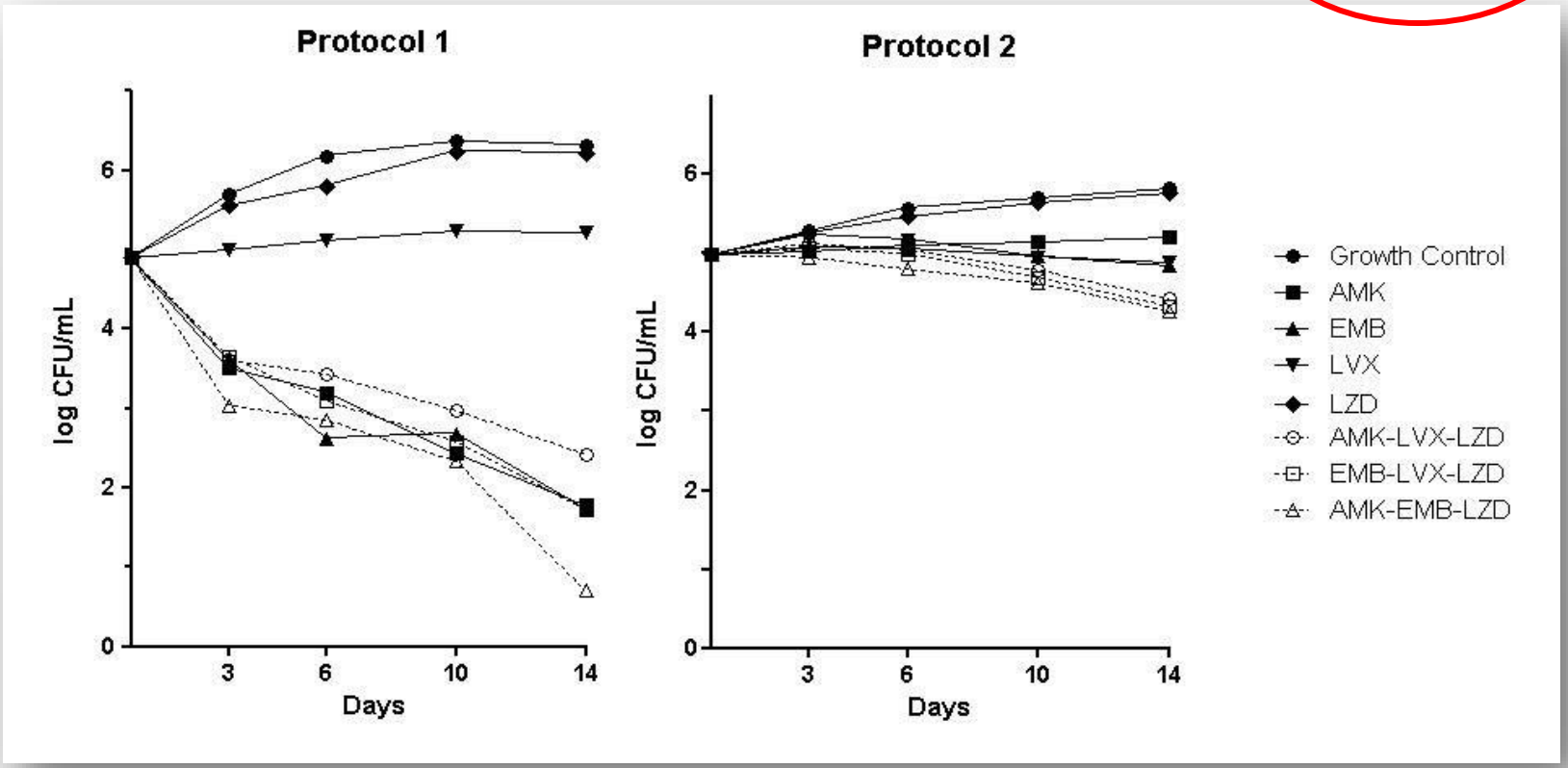


Lorian VM. *Antibiotics in Laboratory Medicine. Fifth edition*

Mètodes *in vitro*: corbes de letalitat

Protocol 1: ½ CMI tots els fàrmacs
Protocol 2. ½ CMI LVX i LNZ i ¼ CMI per EMB i AMK

Combinacions
LEV-LNZ-AMK
LEV-LNZ-EMB
LEV-AMK-EMB

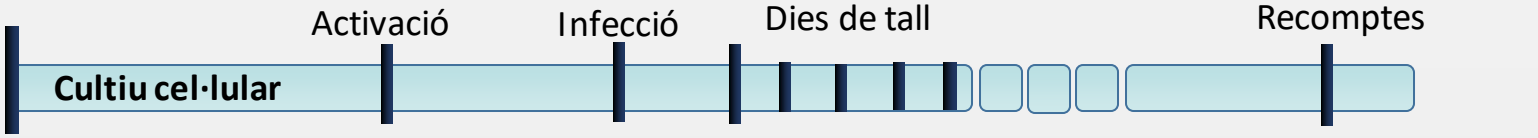


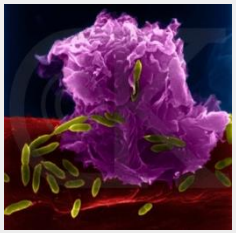
López-Gavín *et al*. *Int J Antimicrob Agents.* 2016 Jan;47(1):97-100.

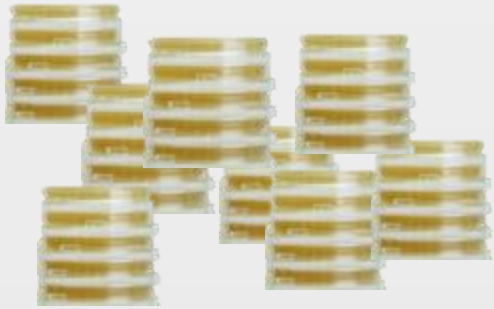
Mètodes *ex vivo*: model d'infecció en macròfags humans

Creixement de la línia cel·lular: THP-1

Renovar el medi amb Atbs nous/dia.

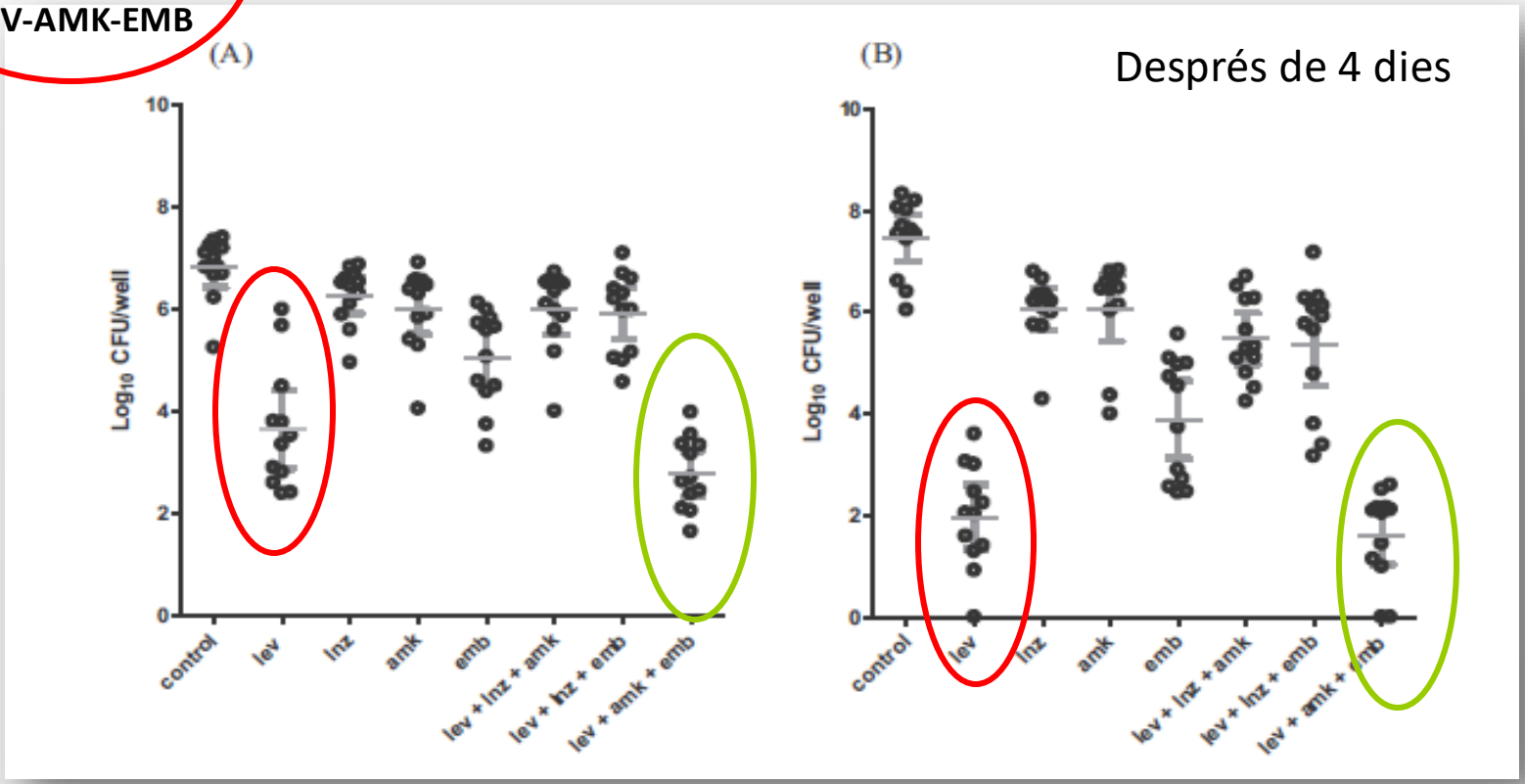






Mètode *ex vivo*: model d'infecció en macròfags humans

Combinacions
LEV-LNZ-AMK
LEV-LNZ-EMB
LEV-AMK-EMB

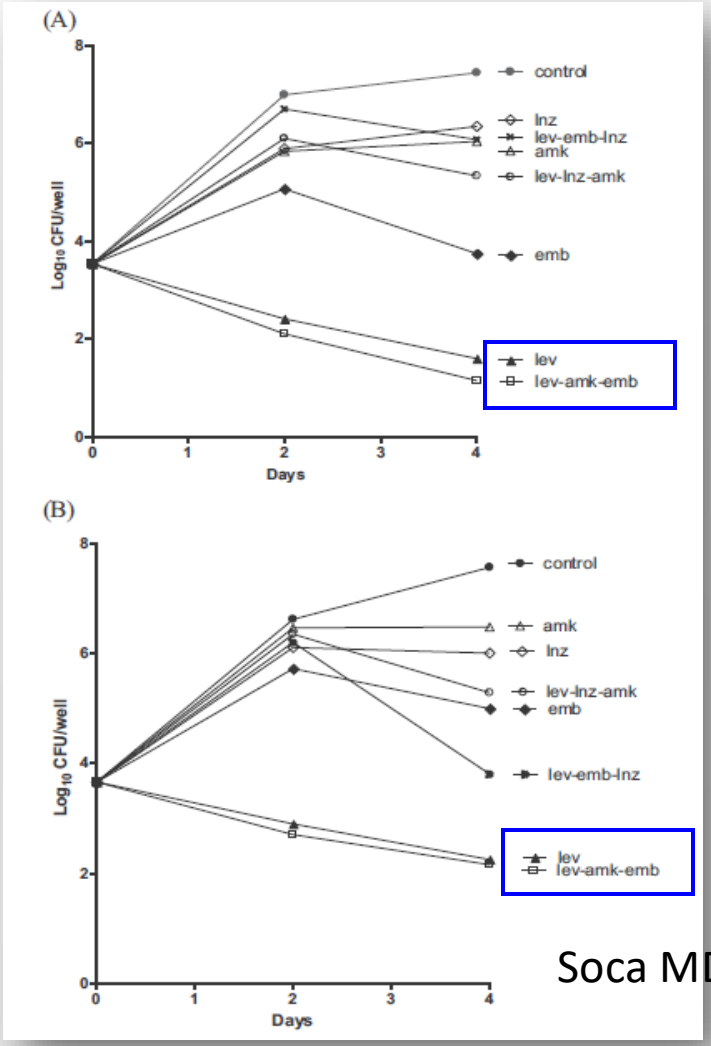


Després de 2 dies

Rey-Jurado *et al*. *Int J Antimicrob Agents.* 2013 Dec;42(6):524-30.

Mètode *ex vivo*: model d'infecció en macròfags humans

Soca sensible



Soca MDR

Table 1
Antimicrobial activity of the drugs studied against the 12 drug-susceptible and multidrug-resistant *Mycobacterium tuberculosis* residing in human macrophages at Day 4.

Drug or drug combination[a]	Reduction in growth[b]
LEV 6 mg/L	5.49 ± 0.51
LNZ 13 mg/L	1.39 ± 0.81
AMK 25 mg/L	1.39 ± 0.63
EMB 5 mg/L	3.57 ± 0.99
LEV–LNZ–AMK	1.98 ± 0.47
LEV–LNZ–EMB	2.09 ± 1.02
LEV–AMK–EMB	5.86 ± 0.47

LEV, levofloxacin; LNZ, linezolid; AMK, amikacin; EMB, ethambutol.
[a] Drugs in the combination were used at the same concentration as the drugs alone.
[b] Mean ± standard deviation of $\log_{10}$ CFU decrease of the 12 isolates studied compared with the control.

Rey-Jurado *et al*. *Int J Antimicrob Agents.* 2013 Dec;42(6):524-30.

Alexandre López-Gavín

Elena Portell Buj

Julià González-Martín

Emma Rey-Jurado

CLÍNIC BARCELONA Hospital Universitari

ISGlobal

UNIVERSITAT DE BARCELONA

Facultat de Medicina i Ciències de la Salut