

BIOTECHNOLOGICAL APPROACH TO DESIGNING A NEW ANTITUBERCULAR VACCINE

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Aim of the project

Test the humoral and the memory T-cell responses towards new antigenic molecules in:

- subjects vaccinated with BCG
- patients with active tuberculosis

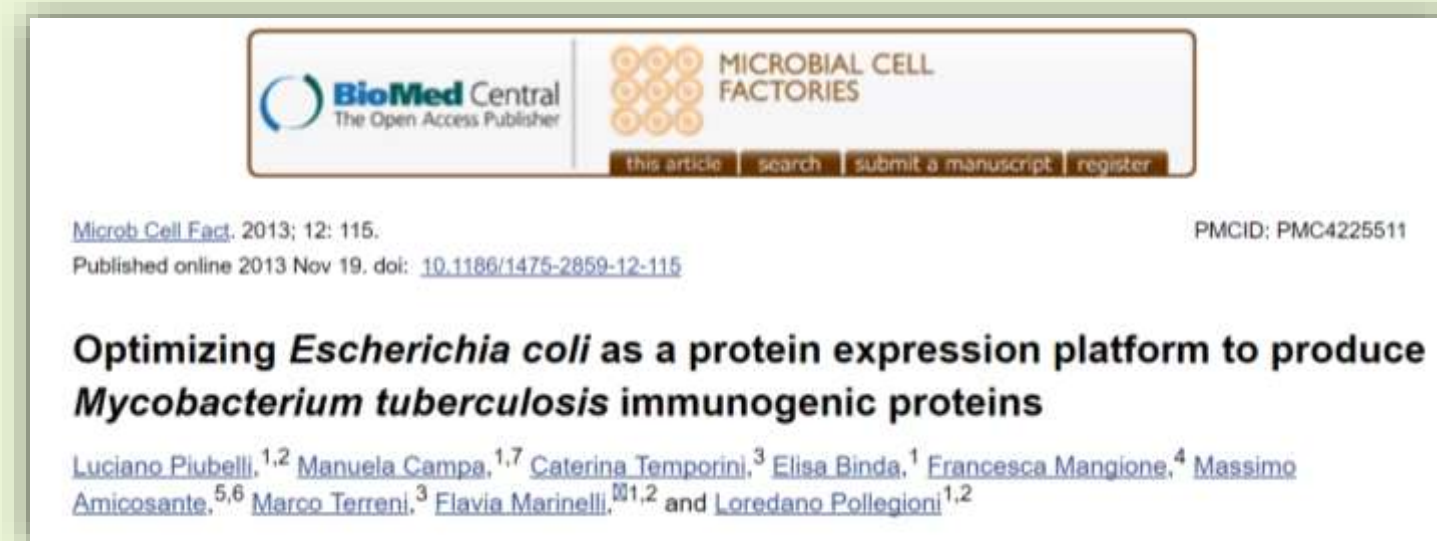
Antigenic complex

Ag85 Complex

- Ag85 A
- **Ag85 B**
- Ag85 C

Esat-6 like

- **TB 10.4**
- TB 10.3
- TB 12.9



The screenshot shows the top portion of a research article page. At the top left is the BioMed Central logo with the tagline 'The Open Access Publisher'. To its right is the 'MICROBIAL CELL FACTORIES' logo, which consists of a grid of orange circles. Below these logos is a navigation bar with buttons for 'this article', 'search', 'submit a manuscript', and 'register'. The article title is 'Optimizing *Escherichia coli* as a protein expression platform to produce *Mycobacterium tuberculosis* immunogenic proteins'. Below the title are the authors' names: Luciano Piubelli, Manuela Campa, Caterina Temporini, Elisa Binda, Francesca Mangione, Massimo Amicosante, Marco Terreni, Flavia Marinelli, and Loredano Pollegioni, with superscripted numbers indicating their affiliations. The journal information 'Microb Cell Fact. 2013; 12: 115.' and the PMCID 'PMC4225511' are also visible.

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Optimizing *Escherichia coli* as a protein expression platform to produce *Mycobacterium tuberculosis* immunogenic proteins

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Studied molecules

Native Proteins

- Ag85B
- TB 10.4



Glycoproteins

- Ag85B-MAN
- Ag85B-MAN-MAN
- TB10.4-MAN
- TB10.4-MAN-MAN



Mutant Proteins

- Ag85B-K23R
- Ag85B-K275R
- Ag85B-K23R/K275R

Subject Enroll Criteria

Subject enrolled	Nr	Criteria
Patients with active TB	24	<ul style="list-style-type: none"> • HIV and HCV negative • Pulmonary infection • Positive culture for <i>Mycobacterium tuberculosis</i> complex • Age under 75 y.o. • 3 weeks of anti-tubercular therapy • Auto-immune diseases negative
Subjects vaccinated with BCG	7	<ul style="list-style-type: none"> • Quantiferon test negative • Auto-immune diseases negative
Negative Control	8	<ul style="list-style-type: none"> • Mantoux test negative • Quantiferon test negative • Auto-immune diseases negative

Tests

Indirect ELISA



Antibody response

10-day
expansion
culture with
proteins



IFN- γ Standard
ELISPOT

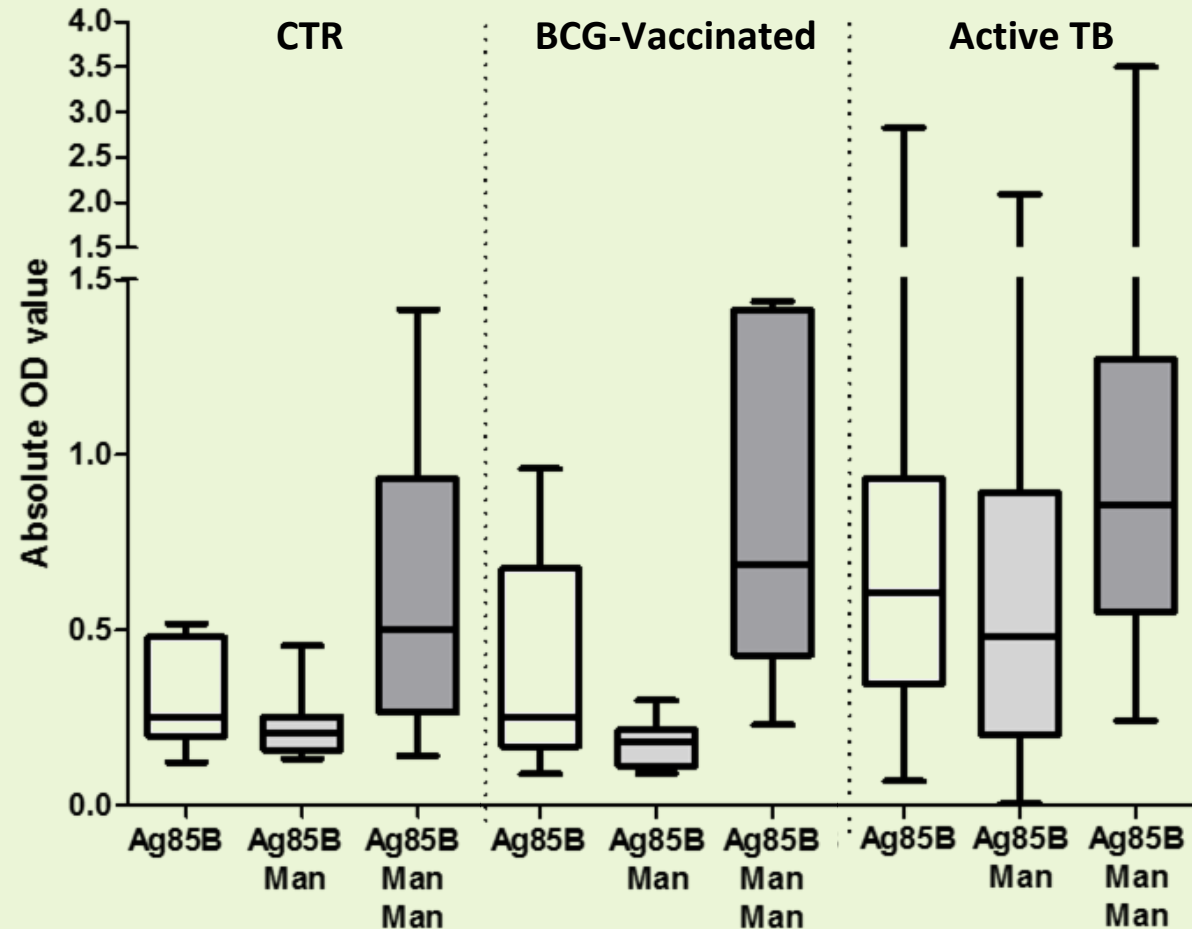


Memory T-cell response

Kashyap R.S., Rajan A.N., Ramteke S.S., Agrawal V.S., Kelkar S.S., Purohit H.J., Taori G.M., and Daginawala H.F. *Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study.* BMC Infect Dis. 2007; 7: 74.

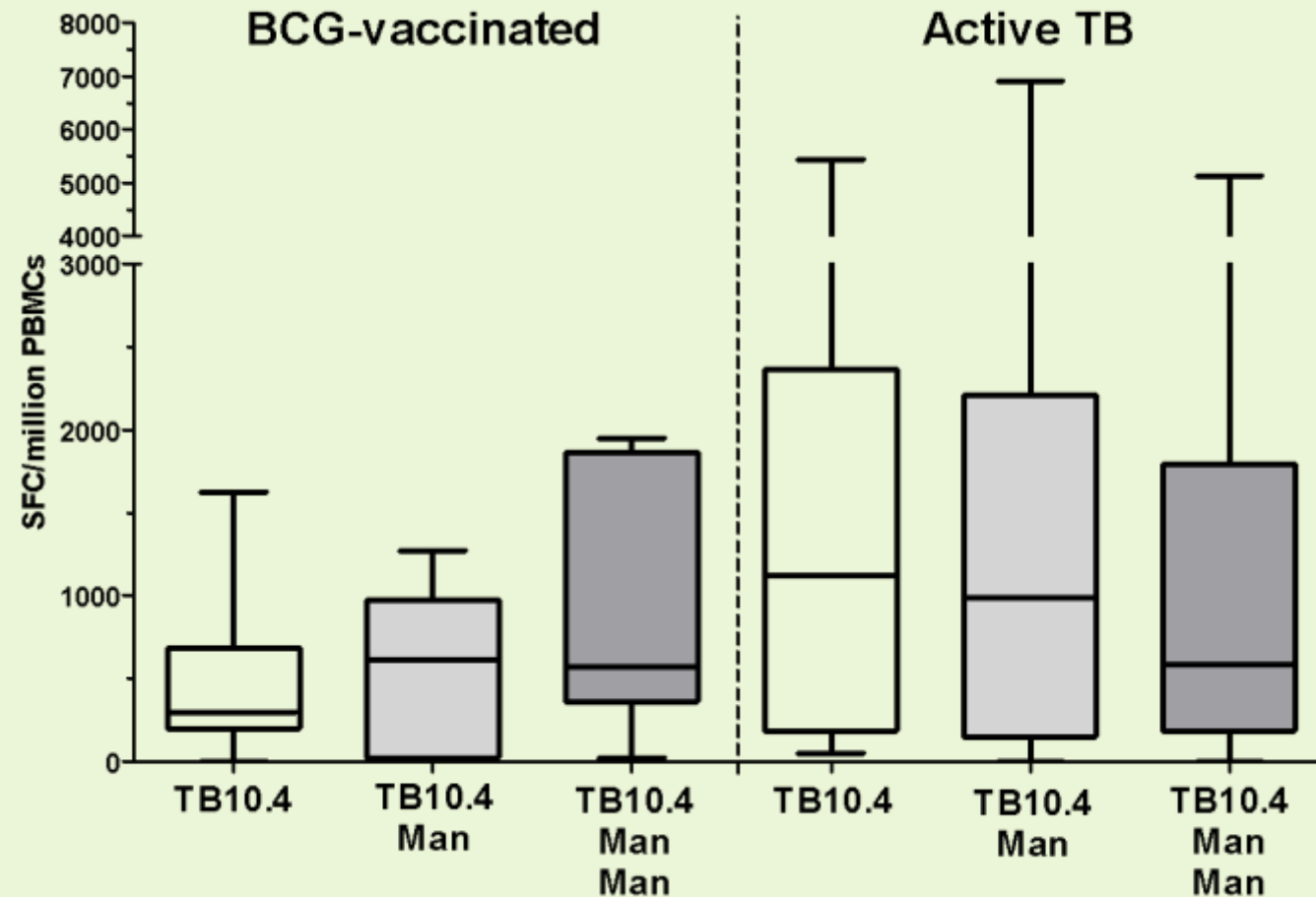
Blunt L., Hogarth P.J., Kaveh D.A., Webb P., Villarreal-Ramos B., Vordermeier H.M. *Phenotypic characterization of bovine memory cells responding to mycobacteria in IFN- γ enzyme linked immunospot assays.* Vaccine. 2015 Dec 16;33(51):7276-82.

Ag85B vs. Ag85B Glycoproteins *Antibody response*



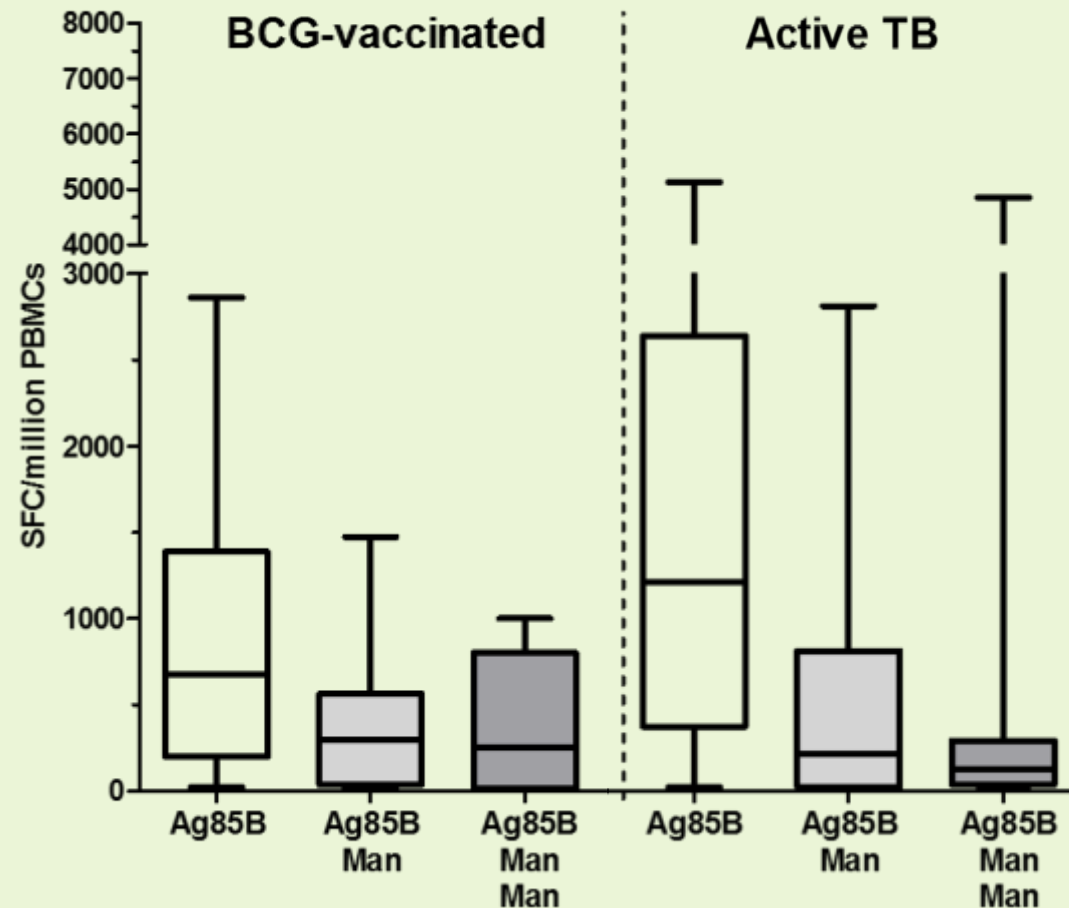
TB10.4 Glycoproteins

Memory T-cell response

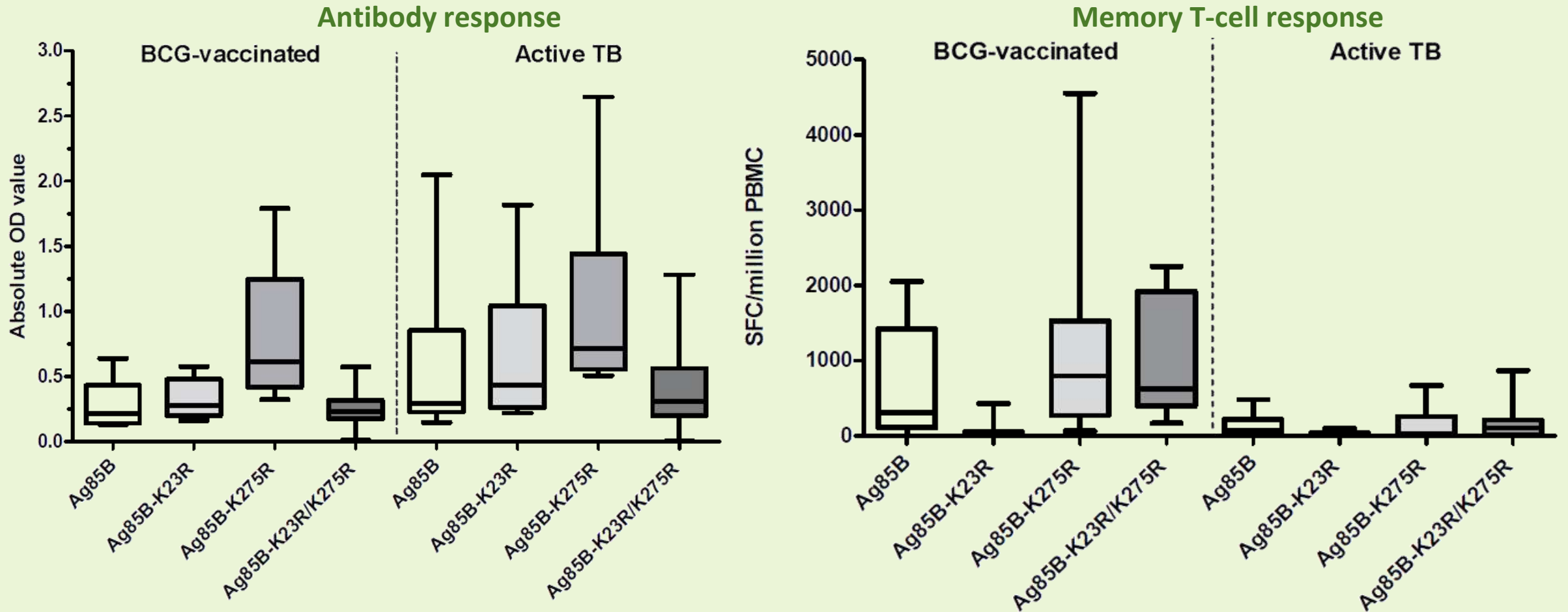


Ag85B Glycoproteins

Memory T-cell response



Ag85B vs. Mutant variants



Conclusions

TB 10.4 glycoproteins



No immunogenic effects

Ag85B glycoproteins



Variable effects

Ag85B-MAN-MAN

Ag85B-K275R and

Ag85B-K23R/K275R



Increased responses

Next step: Mutant Proteins Glycosilation

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Caterina Temporini

Sara Tengattini

“A spoonful of sugar helps the medicine go down”

Mary Poppins



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for your
attention