**TARGET Report**
Experiment in guinea pigs carried out in the laboratory of Dr. David McMurray with Dr. Lan Ly at Texas A&M University using mutants obtained from Dr. Carl F. Nathan and colleagues. Histopathological analysis performed by Dr. Paul Converse at Johns Hopkins University.

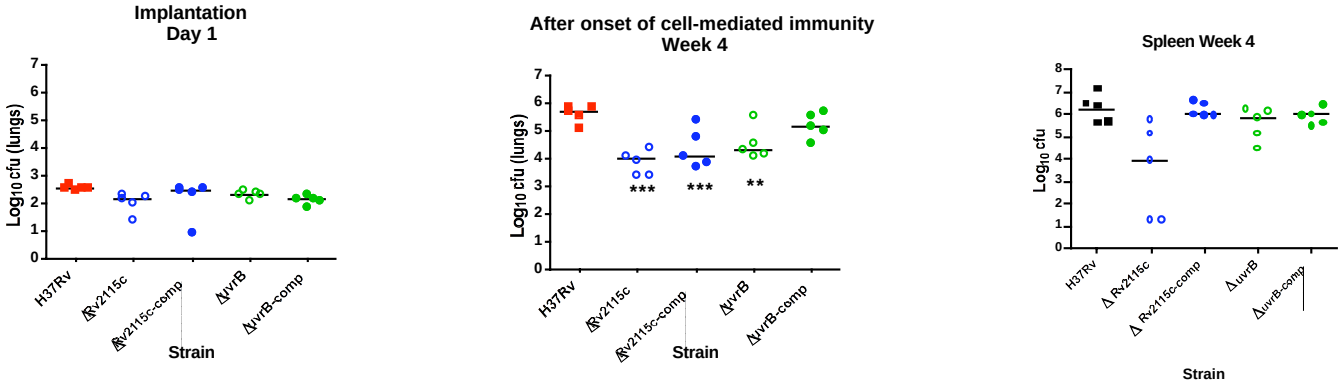
**Strains**

1. H37Rv
2. H37Rv Δ*mpa* (Rv2115c)
3. H37Rv Δ*uvrB*
4. H37Rv Δ*mpa* (Rv2115c) complemented
5. H37Rv Δ*uvrB* complemented

**Goals:**

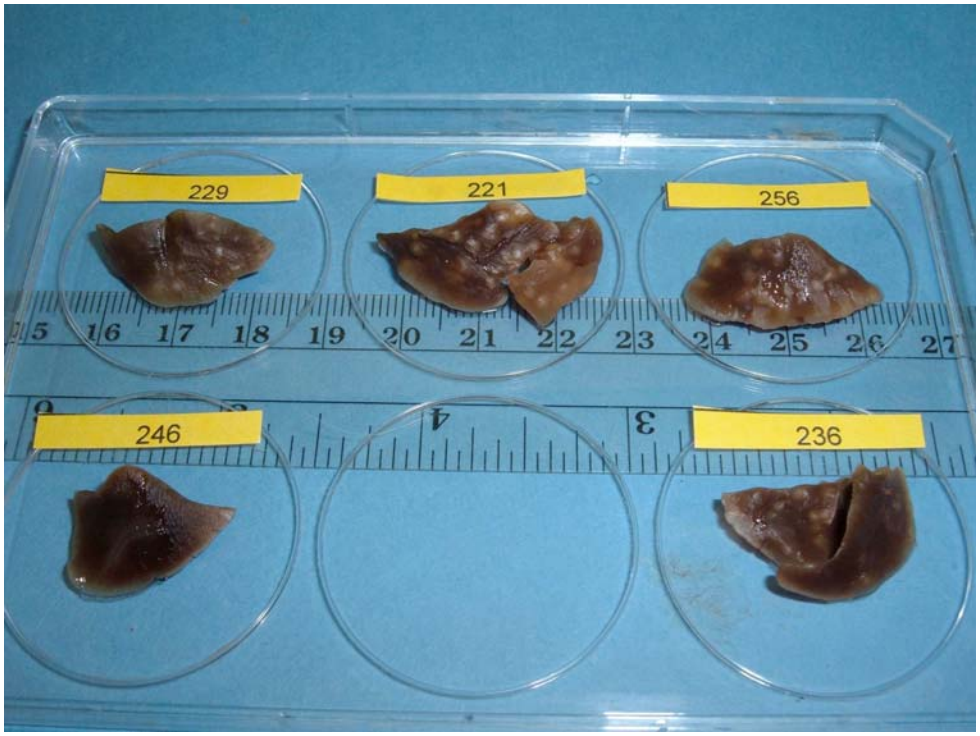
- Assess bacterial burden on day 1 after infection in entire lung (5 lobes)
- Assess bacterial burden on week 4 after infection in lung and spleen
- PPD skin test at 4 weeks after infection
- Lung histopathology at 4 weeks after infection

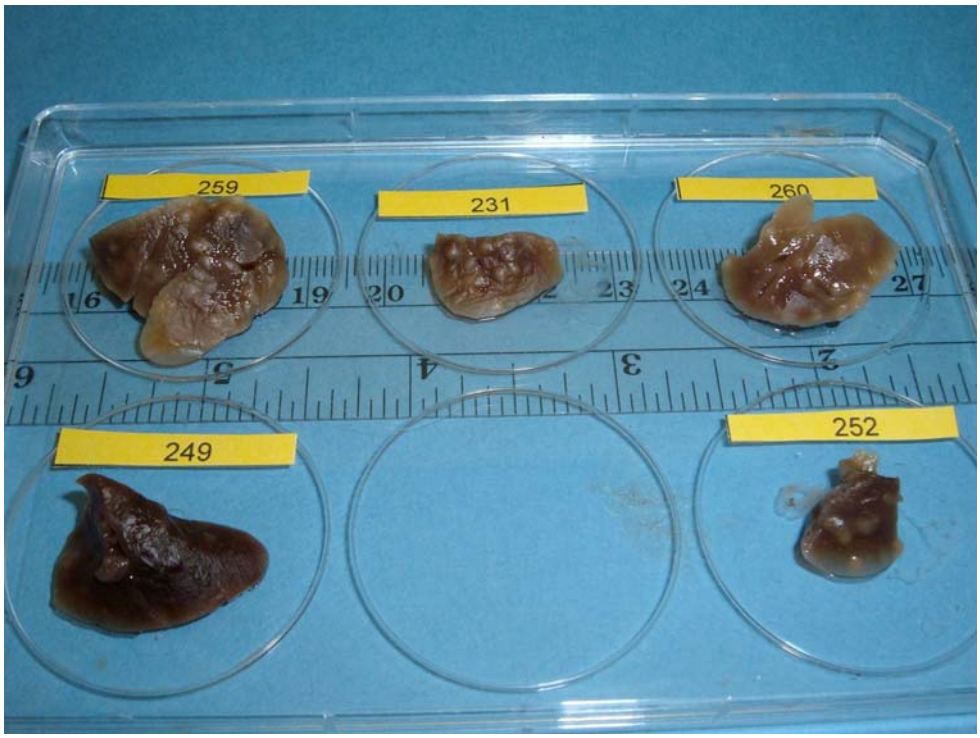
**Day 1 and Week 4 results after aerosol exposure to $10^7$ bacilli/ml**




The day 1 results indicate comparable exposure. There is reduced growth by week 4 compared to H37Rv in all the mutant strains. The differences are statistically significant for Δ*mpa* (Rv2115c) and its complement (both $p<0.001$ by 2-ANOVA) and also for Δ*uvrB* ($p<0.01$) but not Δ*uvrB*-complemented. There is consistently greater variability in the week 4 results than in the day 1 results, possibly reflecting the fact that the guinea pigs are an outbred strain and there may be differences in cell-mediated immunity between animals. In this experiment, there are not significant differences between the Δ*uvrB* and wild-type or complemented strain in the number of bacteria that have disseminated to spleen. The Δ*mpa* (Rv2115c) mutant shows much greater variability and, in two guinea pigs, the number of bacteria is below the detection limit.

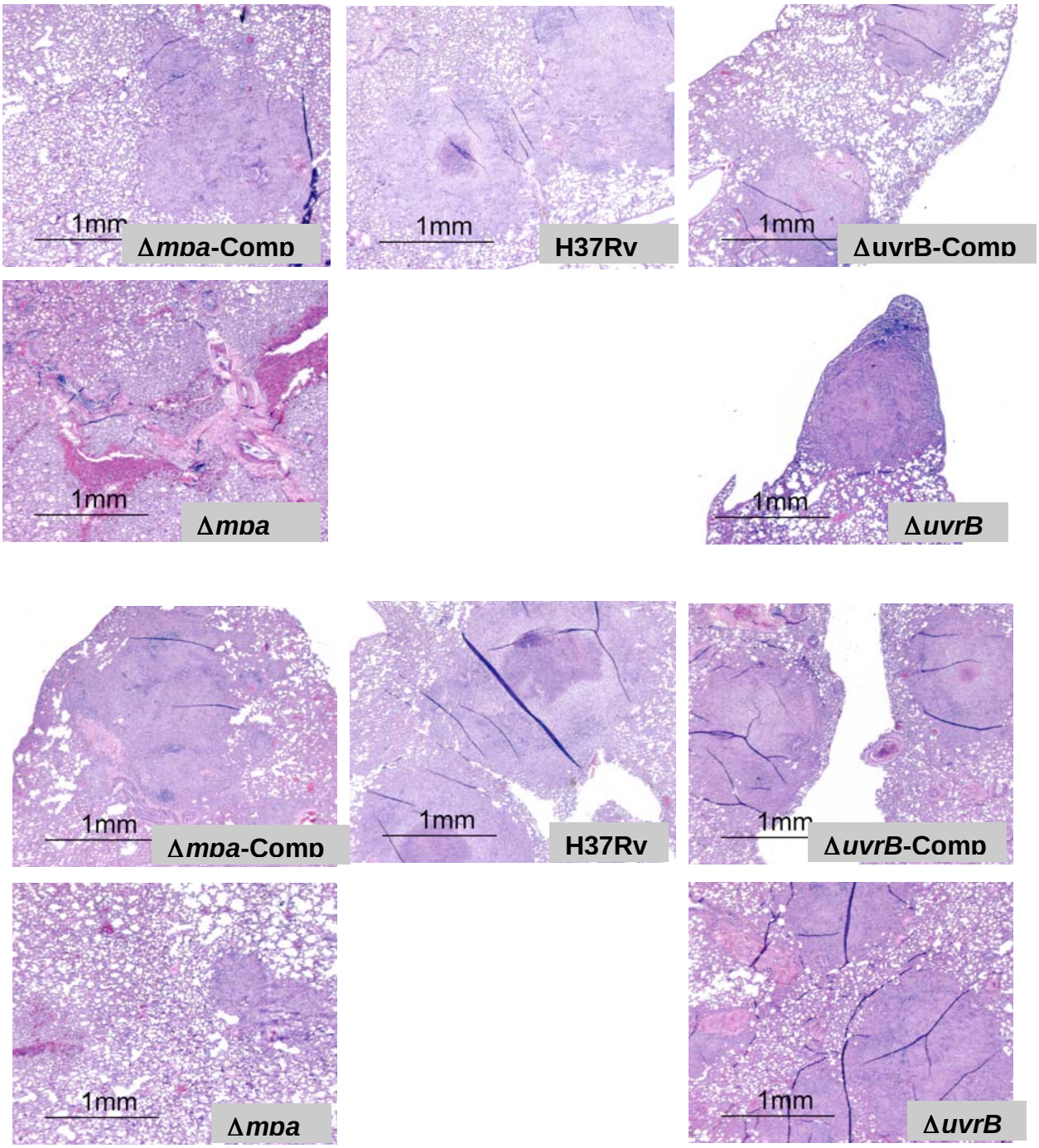
**Gross Lung Pathology at 4 weeks after infection:**




**Key: Top Row each photo, left to right, Δ*mpa*-Comp; H37Rv; Δ*uvrB*-comp**
**Bottom row each photo: left, Δ*mpa*; right, Δ*uvrB***

**Lung Histopathology Week 4:**



**Lung Histopathology**

Two approaches were used to assess the sections (two sections by each method).

1. Sections were measured, in a blinded fashion, using Image Pro Plus software, followed by measurements of granulomatous lesions. Two sections were evaluated for each strain. A third section was included for the Δ*uvrB* complemented strain. Wild-type and complemented strains had the highest percentage of granulomatous lesions while all of the mutants (open symbols), with one prominent exception, induced low percentages of granulomatous lesions.
2. Again, in a blinded manner, for each slide the approximate number of low power (20x) microscopic fields was determined. The number of granulomas was counted and the number of granulomas per microscopic field was calculated. These data plus the extent of caseation was evaluated to arrive at a subjective/semi-quantitative score from 0-4 for each section, using criteria roughly similar to those described by Palanisamy *et al.* Tuberculosis 88:295-306 (2008).

**Histopathology Scores**

Method 1:

| Strain | % granuloma |
|---|---|
| H37Rv | 29.0±20.8 |
| Δ*mpa* | 2.6±1.8 |
| Δ*mpa*-Comp | 11.5±4.2 |
| Δ*uvrB* | 12.1±12.5 |
| Δ*uvrB*-Comp | 12.0±4.2 |

Method 2:

| Strain | # granulomas | # fields | # granulomas/field | Score (0-4) |
|---|---|---|---|---|
| H37Rv | 13.5±13.4 | 1.4±0.9 | 4.36±2.3 | 2.3±0.7 |
| Δ*mpa* | 1.0±1.4 | 1.3±0.4 | 0.7±0.9 | 0.6±0.4 |
| Δ*mpa*-Comp | 14.0±8.5 | 2.25±1.1 | 6.0±0.9 | 1.8±0.6 |
| Δ*uvrB* | 11.5±2.1 | 1.7±0.5 | 7.0±0.7 | 1.4±0.0 |
| Δ*uvrB*-Comp | 8.5±2.1 | 1.5±0.0 | 5.7±1.4 | 1.8±0.3 |

**PPD Skin test at 4 weeks**

| Strain | Mean Diameter (mm) | SEM |
|---|---|---|
| H37Rv | 16.2 | 1.36 |
| H37Rv Δ*mpa* (Rv2115c) | 11 | 0.55 |
| H37Rv Δ*uvrB* | 13 | 2.07 |
| H37Rv Δ*mpa* (Rv2115c) complemented | 12 | 0.84 |
| H37Rv Δ*uvrB* complemented | 14 | 2.30 |

**Interpretation**

There is somewhat lower multiplication in the lungs by the Δ*mpa* ($p<0.001$) and Δ*uvrB* ($p<0.01$) mutants. The Δ*mpa* mutant also appears to induce less granulomatous pathology by both methods of assessment, while the Δ*uvrB* mutant and both complemented strains are not very different histopathologically from the H37Rv wild-type. The Δ*mpa* mutant may have a slight defect in its capacity to disseminate from the lung, but there is animal-to-animal variability. All strains induced a delayed-type hypersensitivity response as assessed by reactions to veterinary grade tuberculin.