

DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE SPECIFIC RECOGNITION OF THE WILD TYPE FORM OF GLUTATHIONE S-TRANSFERASE OMEGA I

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Introduction

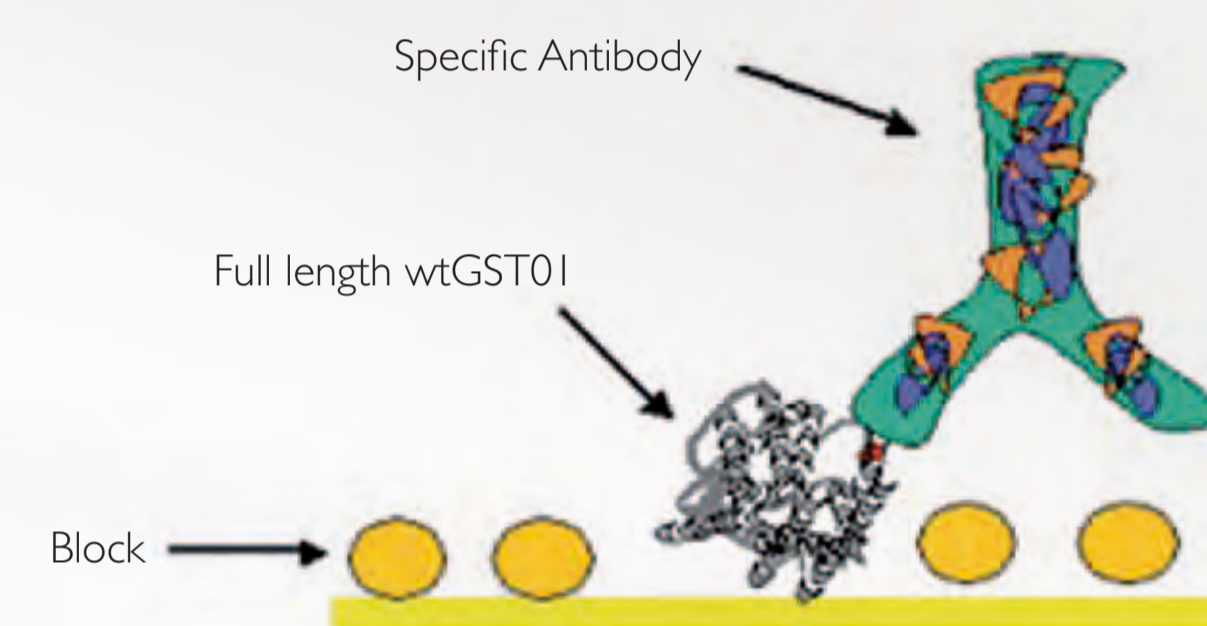
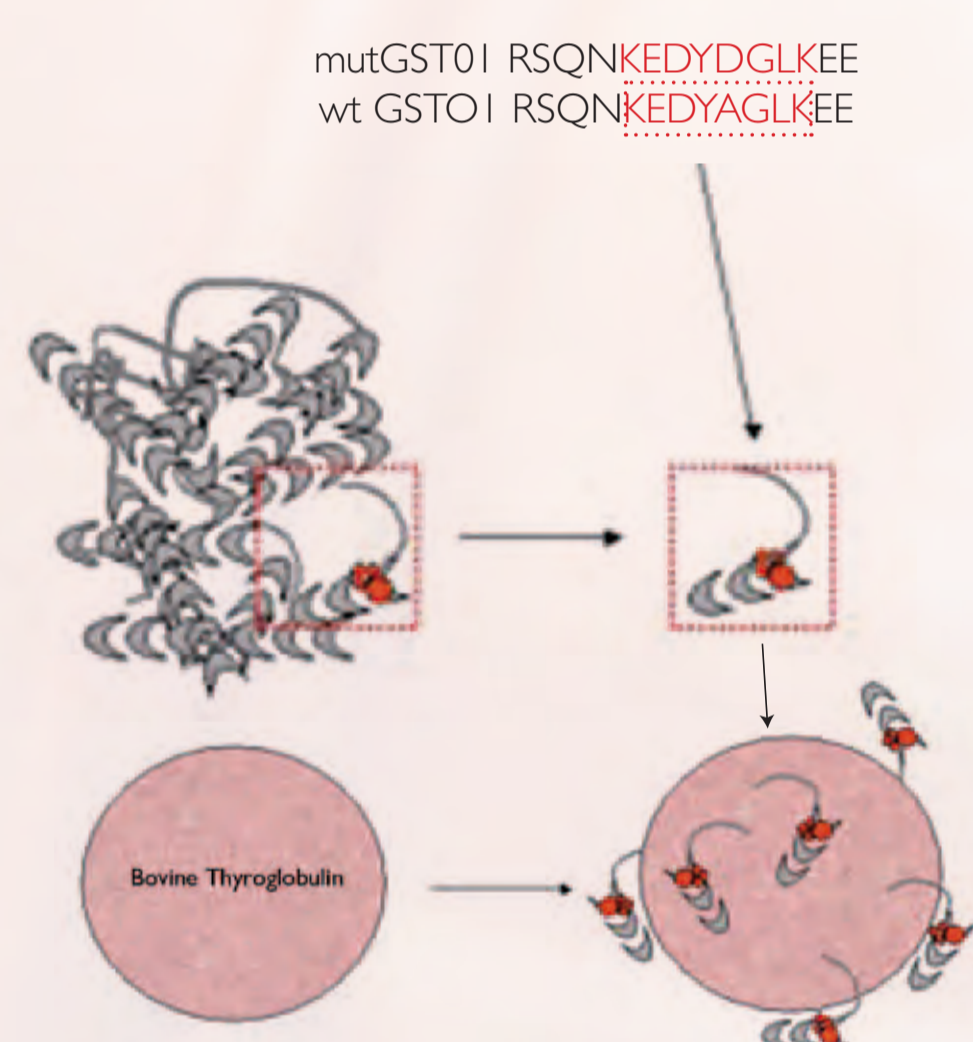
Glutathione S-transferase omega I (GSTO-I) exists as both a wild-type (wt) and a mutant (mut) enzyme due to a polymorphism in a single amino acid Ala140Asp. ⁽¹⁾ The role that this gene product plays in connection with a range of disease conditions has been investigated. ^(2,4) An antibody capable of specifically detecting wtGST would be of clinical

significance to further elucidate the role that wtGSTO1 plays in disease states. The aim of this work was to develop a highly specific monoclonal antibody, which will be used as a tool in the development of clinical research and clinical immunoassays.



Methodology

- Sheep were immunized with a motif, which housed the single amino acid difference between wtGST and mutGST conjugated to Bovine thyroglobulin (BTG) as a carrier. Lymphocytes were collected and fused with heteromyeloma cells.



- The antibodies were purified and evaluated by ELISA. Absorbances were read at 450nm.

Assay Evaluation Parameters

- The calibration curve was generated with the analyte as standard in a sandwich assay using two antibodies. The capture antibody is specific for wtGSTO1. The detecting antibody-conjugated to horseradish peroxidase is generic for both wtGSTO1 and mutGSTO1.

- Specificity: the specificity, expressed as %cross reactivity (%CR) was calculated as follows:
Two samples were generated:
TEST: A mid range calibrator with 316.5ng/ml wtGSTO1 was spiked with 1000ng/ml of mutGSTO1
TARGET: A mid range calibrator with 316.5ng/ml wtGSTO1

Their corresponding concentrations were calculated from the calibration curve and input into the following calculation.

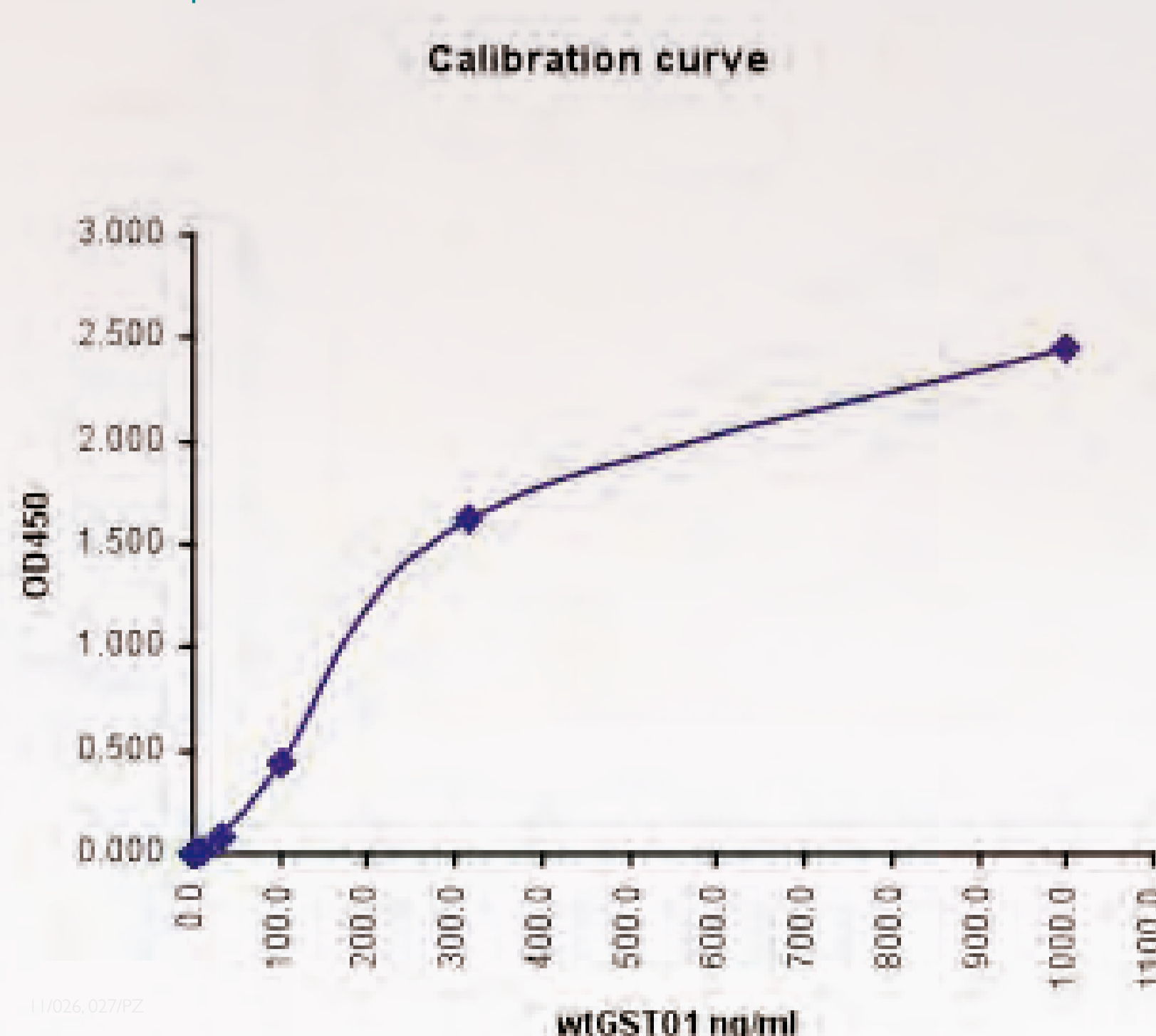
$$\left(\frac{\text{Conc. of TEST [ng/ml]} - \text{Conc. of TARGET [ng/ml]}}{\text{Level of cross reactant [ng/ml]}} \right) \times 100$$

- Intra-assay precision was determined from the results of three replicates at different concentration levels within the same run. Results were expressed as %CV values.

- Supernatants from the resulting hybridomas were screened for the presence of generic antibody using ELISA based assays coated with full length protein. Positive hybridomas were cloned to produce stable monoclonal hybridomas.

Results

Results corresponding to the initial antibody evaluation are presented



	Calculated concentration (ng/ml)	%CR
TEST (wt + mut)	307.5	<1%
TARGET (wt)	316.45	

Intra-assay precision: %CV <15% for different concentration levels

Conclusion

Data indicates that the monoclonal antibody generated recognizes specifically the wild type form of glutathione S-transferase omega I and creates a new clinical tool for its quantitative and qualitative detection for studies of its role in disease states.

REFERENCES

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2. Sunanta Chariyalertsak et al. Role of glutathione S-transferase omega gene polymorphisms in breast-cancer risk. *Tumori*, 2009, 95: 739-743
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