## 141 C57BL/6 MICE CURED OF B78 MELANOMA FREQUENTLY DEVELOP ANTIBODIES THAT RECOGNIZE A 4 AMINO ACID BINDING MOTIF (SDTG)

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**Background** We utilized a high-density peptide array, to identify linear peptide sequences of protein-targets recognized by anti-tumor antibodies produced in mice cured of melanoma following immunotherapy.

**Methods** Mice bearing B78 melanomas were treated with a combination immunotherapy [local radiation therapy + intratumoral immunocytokine (anti-GD2 mAb linked to IL2)] that induced an '*in situ* vaccine' effect (ISV), enabling mice to be cured of their tumors with long-term immune memory.[1] Naïve (prior to tumor injection) and immune (post-rechallenge/after cure) sera were collected from these mice. Sera were tested using a whole-proteome peptide-array of stacked 16-mer peptides.[2] Specific antibody-binding sites and recognized epitopes were identified, using an algorithm [HERON] that ranks candidates recognized by immune sera but not by sera from naïve mice.[3] For proteins highly recognized by immune sera, RNA-seq was used to assess differential expression of their genes in tumor vs normal and treated vs untreated tumor tissues.

**Results** We identified many epitopes recognized selectively by sera of immune mice. Among the 100 top epitopes ranked by their binding strength to immune sera from at least 50% of the immune mice tested, we found a 4-amino acid (aa), 'SDTG' motif that was recognized in >60 of these epitopes. However, not all peptides containing this SDTG motif exhibited binding. The aa located just before and the 2 aa just following the SDTG motif play a role in binding. Using an unrelated cohort of mice, we were able to show binding of some additional immune mouse samples to selected peptides containing the SDTG motif. The antibody to the SDTG motif is not caused by the transfection used to induce GD2 expression in B78 cells; as mice cured of the parental B78-H1 cell line (not expressing GD2) also showed reactivity to some SDTG-containing peptides. The immunocytokine does not contain SDTG in its linear sequence. RNAseq analysis revealed upregulation of genes with highly recognized SDTG-containing peptides in radiated vs. untreated B78 cells.

**Conclusions** This SDTG motif might be an important piece in anti-tumor immunity to B78 melanoma. We are further investigating what causes binding to the motif, and if the antibodies against it might have been induced by sensitization to one specific tumor protein, or possibly several proteins. The presence of antibodies against this motif might be a biomarker to predict response to our ISV regimen and this approach might be used for analyses of other effective immunotherapy treatments.

### REFERENCES

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