PREVALENT BINDING MOTIF IN C57BL6 MICE CURED OF B78 MELANOMA VIA IMMUNOTHERAPY

Anna Hoefges*, Nicholas Mathers, Sean Mcilwain, Richard Pinapati, Bradley Garcia, Jigar Patel, Amy Erbe, Irene Ong, Paul Sondel. University of Wisconsin Madison, Madison, WI, USA; Nimble Therapeutics, Inc, Madison, WI, USA

Background Using a high-density peptide array, we assessed potential protein-targets for antibodies detected in mice cured of melanoma through a combined immunotherapy regimen. Our goal was to determine the linear peptide sequences recognized by anti-tumor antibodies produced in mice cured of melanoma following immunotherapy.

Methods Mice bearing B78 melanoma tumors were treated with a combination immunotherapy (local radiation therapy + intratumoral anti-GD2 mAb linked to IL2) capable of inducing an ‘in situ vaccine’ effect (ISV), enabling mice to be cured of their tumors with long-term immune memory. Naïve (prior to tumor injection) and immune (post-rechallenge/after cure) sera were collected from these mice. Using flow cytometry, immune sera showed strong antibody-binding against B16 (parental cell line of B78) and B78. These sera were then used on a Nimble Therapeutics’ whole-proteome peptide-array to determine specific antibody-binding sites, and data were analyzed using a dynamic programming method that scans adjacent peptides to determine whether a peptide is bound by antibodies. Epitopes were selected if peptides were bound using immune sera but not bound, or significantly less, with the sera from naïve mice.

Results We identified many binding epitopes only present in immune mice. Among the epitopes found, we noticed a repeating motif consisting of 4 amino acids that made up over 60% of epitopes that are present in at least 50% of mice in our most restrictive binding category. However, the 4 amino acid long motif is not the only reason for binding as some peptides including this motif do not show binding. The amino acid (aa) sequence before and the 2aa sequences following the motif seem to be important for binding. Using an unrelated cohort of mice we were able to show binding of some additional immune mouse samples to selected peptides containing the identified motif.

Conclusions We think that this motif might be an important piece in anti-tumor immunity to B78 melanoma. We are further investigating what causes binding vs no binding to the motif, and if the antibodies against it originated at one specific site vs multiple sites. The presence of antibodies against this motif might be a useful biomarker to predict response to our ISV regimen and might have the potential to be used for other immunotherapy treatments.

REFERENCE
