

## **An optimized T cell epitope forecast approach for the identification of new human antigenic peptides derived from tumor-expressed splice variants**

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The analysis of the human genome/transcriptome shows that ~ 60% of all human pre-mRNAs are alternatively spliced; nevertheless, only few tumor-specific splice variants have been already reported. It is unclear how widespread it is and to what extent aberrant alternative splicing contributes to tumorigenicity. Likewise, it is not clear the extent to which it might be a source of defective ribosomal products. We took a bioinformatic approach to identify splice variants of the Melan-A gene.

The next step consists of identifying putative antigenic peptides derived from these splice variants. Since it is known that the C-termini of antigenic peptides are directly produced by the proteasome, the peptides encoded by one splice

variant of Melan-A are synthesized chemically and digested in vitro with purified proteasomes. The resulting fragments are identified by mass spectroscopy to detect cleavage sites. Using this information and based on the available anchor motifs for defined HLA class I molecules, putative antigenic peptides could be predicted. Their relative affinity for HLA molecules was confirmed experimentally with functional competitive binding assays and they were used to search patients' peripheral blood lymphocytes for the presence of specific cytolytic T lymphocytes (CTLs). CTL clones specific for a splice variant of Melan-A could be isolated; although they recognized peptide-pulsed cells, they failed to lyse melanoma cells in functional assays of antigen recognition.