Cancer therapies attempt to exploit differences between tumor and normal cells. As cancer cells proliferate rapidly, many chemotherapies target rapidly dividing cells, causing toxicities for proliferating non-cancerous cells. As cancer is a disease caused by DNA aberrations,1 mutations provide another difference between tumor caused by DNA aberrations,1 mutations between tumor and normal cells.

Tumors contain a large number of mutations, ranging from the 10s to the 100s, that are unique to the tumor relative to the normal cells. Causative "driver" mutations shared by a subpopulation of patients can sometimes be targeted by small molecule inhibitors, such as the BRAF V600E mutation.3 However, in the vast majority of cancer types there are no highly penetrant mutations. Rather, 95% of the mutations in a patient tumor appear to be unique to that tumor.4 Thus, the tumor mutanome may offer a large number of potential targets for personalized vaccine therapies. Our recent work,6 summarized in Figure 1, addressed following key questions: What method is suitable for identifying tumor mutations? Are they immunogenic? Does immunization with mutation-encoding antigens provide a survival benefit? The "next-generation sequencing" (NGS) technology enabled us to profile cancer and normal cells to identify somatic mutations. Comparing mouse B16F10 melanoma cells to the parental reference C57BL/6 cells, we found 1,392 point mutations in coding regions. 962 cause non-synonymous protein changes. Using our RNA vaccine platform, we confirmed endogenous expressed somatic mutations. Of the mutations we tested, one third were immunogenic. Immunization conferred in vivo tumor control, qualifying mutated epitopes as source for effective vaccines.

Next generation sequencing enables identification of immunogenic tumor mutations targetable by individualized vaccines. In the B16F10 melanoma system as pre-clinical proof-of-concept model, we found a total of 563 non-synonymous expressed somatic mutations. Of the mutations we tested, one third were immunogenic. Immunization conferred in vivo tumor control, qualifying mutated epitopes as source for effective vaccines.
Observed T-cell frequencies were similar to the frequencies induced by the immunodominant Trp2\(^{180-88}\) epitope, demonstrating that many of the mutations are immunodominant epitopes of B16 melanoma. This is the first experimental data establishing the breadth of the immunogenicity of the tumor mutanome. Our findings match previous in silico predictions suggesting 30 to 50% of non-synonymous mutations are immunogenic.\(^8\)

We examined whether immunization with mutation-coding peptides would translate into a tumor survival benefit. By prophylactic vaccination complete tumor protection in 40% of the mice was achieved. In therapeutic models, we observed a remarkable growth inhibition induced by mutation-coding peptide immunization. These results demonstrate that vaccination against a single mutation encoding sequence is able to induce substantial anti-tumoral effects. To avoid tumor escape due to evolution under immunoediting pressure,\(^9\) multiple mutations could be targeted. Noteworthy, we found no correlation between immunogenicity and potential oncological function, structural features or subcellular localization of the encoded protein. Thus, regardless of whether the mutation is a “driver” or “passenger” mutation, its utilization in a vaccine appears to provide an anti-tumor benefit.

In conclusion, in a pre-clinical model, we successfully demonstrate a blueprint process to identify somatic mutations, select mutations for an individual therapeutic vaccine, and immunize to provide an anti-tumor impact. Our data shows that the T-cell druggable mutanome is substantial and can be exploited for patient benefit using individualized therapeutic cancer vaccines.

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