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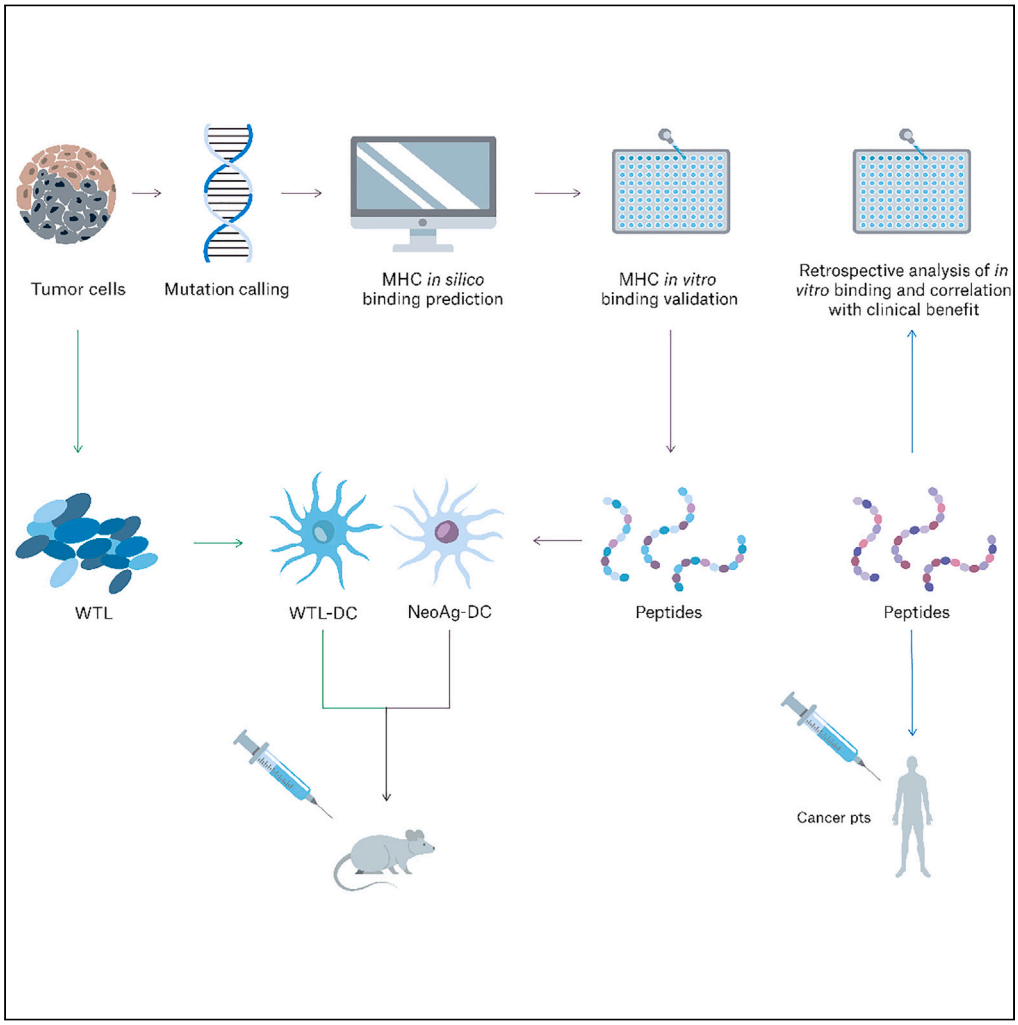

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Highlights

Antigen selection and prioritization are a crucial factor for vaccine development

In vitro peptide-MHC binding affinity improves the ranking of neoantigens

Peptide immunogenicity improves the ranking of neoantigens

Whole-tumor-lysate vaccines represent a rich source of personalized antigens

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Cancer vaccines based on whole-tumor lysate or neoepitopes with validated HLA binding outperform those with predicted HLA-binding affinity

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SUMMARY

Antigen selection and prioritization represent crucial determinants of vaccines' efficacy. Here, we compare two personalized dendritic cell-based vaccination strategies using whole-tumor lysate or neoantigens. Data in mouse and in cancer patients demonstrate that peptide vaccines using neoantigens predicted on the sole basis of *in silico* peptide-major histocompatibility complex (MHC) binding affinity underperform relative to whole-tumor-lysate vaccines. In contrast, effective *in vitro* peptide-MHC binding affinity and peptide immunogenicity significantly improve the prioritization of tumor-rejecting neoepitopes and result in more efficacious vaccines.

INTRODUCTION

Dendritic cell (DC)-based vaccination is currently considered as a valid therapeutic option for cancer treatment in the adjuvant setting. Monocyte-derived autologous DCs loaded with tumor antigens have demonstrated limited toxicity and potent immunogenicity yet with clinical benefits remaining suboptimal, leaving room for further improvements.[1]

Despite objective and durable responses recently reported with neoantigen vaccines,[2,3] neoantigen prioritization (*i.e.*, which peptides containing a non-synonymous cancer-specific mutation are most likely to be immunogenic) remains challenging.[4,5] On the other hand, whole-tumor lysate (WTL) constitutes a valid alternative source of cancer antigens that circumvents the need for antigen identification and validation. Recent work by our group has demonstrated that WTL-DC vaccines (WTL-DC vax) were able to boost pre-existing T cell responses and induce *de novo* neoantigen-specific T cell responses in ovarian cancer patients, associated with clinical benefit.[6,7] However, the relative efficacy of WTL- *versus* private neoantigen-based vaccines has never been comprehensively investigated.

Here, we addressed this issue in mouse models and in cohorts of vaccinated ovarian and lung cancer patients. Our investigations show that WTL-DC vax are efficient and outperform DC vaccines pulsed with neoantigen peptides selected exclusively with common *in silico* predictors of peptide-major histocompatibility complex (pMHC) affinity. Furthermore, parameters such as effective *in vitro* pMHC binding or peptide immunogenicity significantly improve the prioritization of tumor-rejecting neoepitopes in both mouse and cancer patients, resulting in improved clinical responses.

RESULTS

Efficacy of WTL-DC vax and neoantigen-based vaccines in melanoma, lung, and ovarian cancer tumor models

To assess WTL-DC vax's efficacy, we vaccinated mice bearing melanoma (B16F10), lung (Lewis Lung Cancer, LLC1),[8,9] and ovarian (ID8) tumors (Figure 1A) and observed significant delays in tumor progression in all models (Figures 1B and 1C). In contrast, no tumor control was observed with unpulsed DC (Figure S1). We next focused on the LLC1 model to compare the efficacy of DCs loaded with either WTL or neoantigen peptides. To this end, 3,106 SNVs were identified and further filtered based on gene expression and distance-to-self score.[10,11] Fifty-eight neoepitope candidates with strong *in silico* human leukocyte antigen

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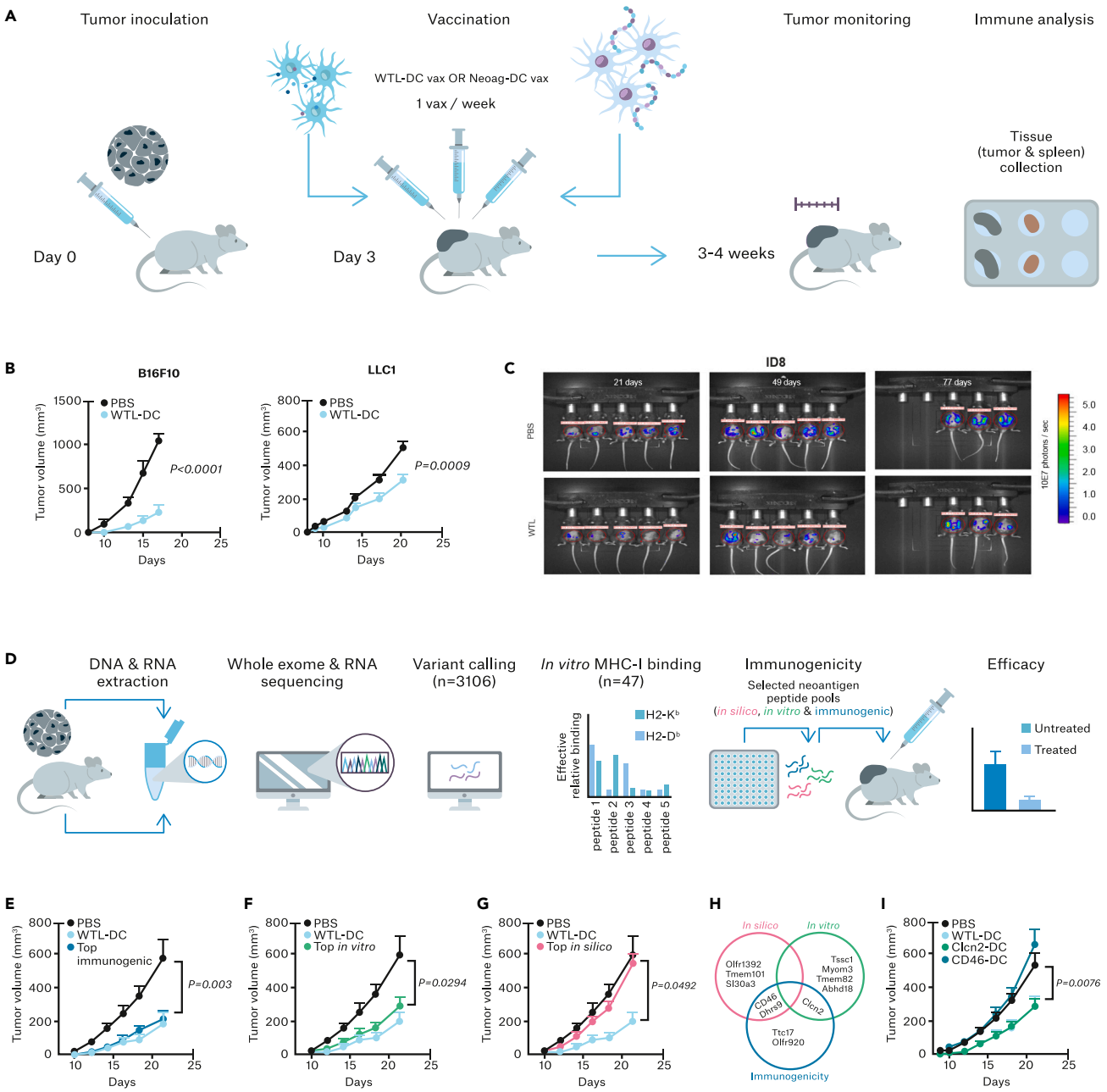

Figure 1. Efficacy of WTL-DC vaccines and neoantigen-based vaccines in melanoma, lung, and ovarian cancer tumor models

(A) Schematic representation of the vaccination pipeline.

(B) Tumor growth curve of mice implanted subcutaneously (s.c.) with either 1 × 10^5 B16F10 (n = 35) or 2x10^5 LLC1 tumor cells (n = 15) and immunized every week for three weeks with WTL-DC intradermally (i.d.).

(C) Bioluminescence measurement of tumor growth in mice (n = 9) implanted intraperitoneally with 5x10^6 ID8 tumor cells and vaccinated i.d. every week for four weeks with WTL-DC. PBS was used as control in (B) and (C).

(D) Schematic representation of the neoantigens discovery pipeline. After DNA and RNA sequencing and neoantigen prioritization, *in silico* predictions to MHC class I alleles were performed using NetMHC4.0 and NetMHCpan3.0. *In silico* predictions were further refined based on the effective *in vitro* binding of *in silico* predicted peptides to H2- D^b and H2-K^b.

(E–G) Tumor growth curves of mice implanted s.c. with 2 x 10^5 LLC1 tumor cells (n = 10) and vaccinated with WTL-DC or the top five peptides from each peptide selection pipeline (*i.e.*, *in silico* prediction, *in vitro* binding affinity, and *in vivo* immunogenicity, Table S1).

Figure 1. *Continued*

(H) A schematic representation of the peptide overlap between prediction pipelines based on the top immunogenic, the top *in vitro*, and the top *in silico* peptides from (E–G).

(I) Tumor growth of mice (n = 10) inoculated with $2x10^5$ LLC1 tumor cells and immunized with WTL-DC vax, CD46-DC, or Clcn2-DC vax. Data report mean ± SEM. Statistical analyses: non-parametric unpaired t-test and one-way ANOVA.

(HLA) binding affinity were then selected using NetMHCpan3.0, out of which 47 peptides demonstrated effective HLA-binding affinity *in vitro*, using a robust pMHC refolding assay (Figures 1D and S2).[12,13] All peptides were also tested for their immunogenicity *in vivo* (Figure S3 and Table S1).

To determine how to best identify tumor-rejection antigens, LLC1 tumor-bearing animals were vaccinated with DCs pulsed with the top five peptides from each peptide selection pipeline (*i.e.*, *in silico* prediction, *in vitro* binding affinity, and *in vivo* immunogenicity) and compared to WTL-DC vax (Figure 1A). DCs pulsed with the top five *in silico* peptides showed no efficacy while DCs pulsed with either the top five immunogenic or the top five *in vitro* peptide binders yielded the same efficacy as that of WTL-DC vax (Figures 1E–1G). As a further validation, peptides re-prioritized with more recent versions of *in silico* predictors (*i.e.*, NetMHCpan.4.1)[10] also failed to limit tumor growth while, conversely, peptides selected with PRIME1.0 (*i.e.*, an immunogenicity predictor)[14] induced the same level of tumor control as that of WTL-DC vax (Figure S4).

We then aimed to identify the individual epitope(s) driving tumor control from the different pipelines. While no peptide was common across all three pipelines, $CD46_{KSYTFFSCSL}$ and $Dhrs9_{FGLINVTPNM}$ were part of both the top *in silico* and immunogenic groups, and $Clcn2_{FAVRNYWRGV}$ was the unique common epitope between the top *in vitro* binders and immunogenic groups (Figure 1H). Immunization with $CD46_{KSYTFFSCSL}$ alone (selected for the higher immunogenicity over $Dhrs9_{FGLINVTPNM}$, Figure S3) demonstrated no efficacy while immunization with $Clcn2_{FAVRNYWRGV}$ alone provided the same level of tumor control as that of WTL-DC vax (Figure 1I). Of note, immunization with $Clcn2_{FAVRNYWRGF}$ (wild-type) did not demonstrate tumor control (Figure S5).

Our data so far demonstrate that WTL vaccination was more effective in controlling LLC1 tumor growth than vaccination with neoantigens selected exclusively with any of the commonly used *in silico* predictors based on pMHC affinity. In contrast, vaccine's efficacy significantly improved when peptides were further selected on the basis of their effective *in vitro* binding or immunogenicity.

To further demonstrate the relevance of effective *in vitro* pMHC binding affinity measurement in identifying clinically relevant neoepitopes, we reanalyzed several independent mouse and human cancer vaccine studies. We first evaluated, in mouse, the effective (*in vitro)* binding of a tumor-controlling neoepitope, $Ccdc85c_{YIRPFETKVK}$, reported as a poor *in silico*-predicted MHC binder.[15] Of interest, the *in vitro* binding affinity of $Ccdc85c_{YIRPFETKVK}$ to H2-Kd was significantly high, *i.e.*, consistent with its clinical efficacy but inconsistent with its *in silico* MHC binding score (Figure 2A). Of note, the *in vitro* binding of the wild-type peptide version of $Ccdc85c_{YIRPFETKVK}$ was also determined and showed limited MHC binding (Figure S6).

Second, we analyzed several neoepitopes identified in a cohort of WTL-DC-vaccinated ovarian cancer patients in order to further evaluate how *in silico* and *in vitro* HLA-binding scores predict immunogenicity (Figure 2B).[6] By focusing on the six patients with detectable neoepitopes, a reanalysis of the *in silico* HLA-binding score (NetMHCpan v4.1) of all immunogenic (n = 31) and non-immunogenic (n = 535) peptides demonstrated no significant differences (Figure S7). To learn more, we determined the *in vitro* HLA-binding score of all immunogenic and of the top 10 best *in silico* binders from non-immunogenic peptides of each patient (methods and Table S2). While neither the *in silico* nor the *in vitro* score was significantly different between immunogenic and non-immunogenic peptides, the relative ranking (thus prioritization) of immunogenic neoepitopes was significantly higher when peptides were ranked based on their *in vitro* binding score rather than their *in silico* score (Figure 2C, left panel). Furthermore, by limiting the total number of peptides per patient to five (mimicking the clinical setting), the proportion of immunogenic peptides was significantly higher when prioritization was based on the *in vitro* score as compared to the *in silico* score (Figure 2C, right panel). In other words, the *in vitro* binding score significantly enriches predictions in immunogenic peptides.

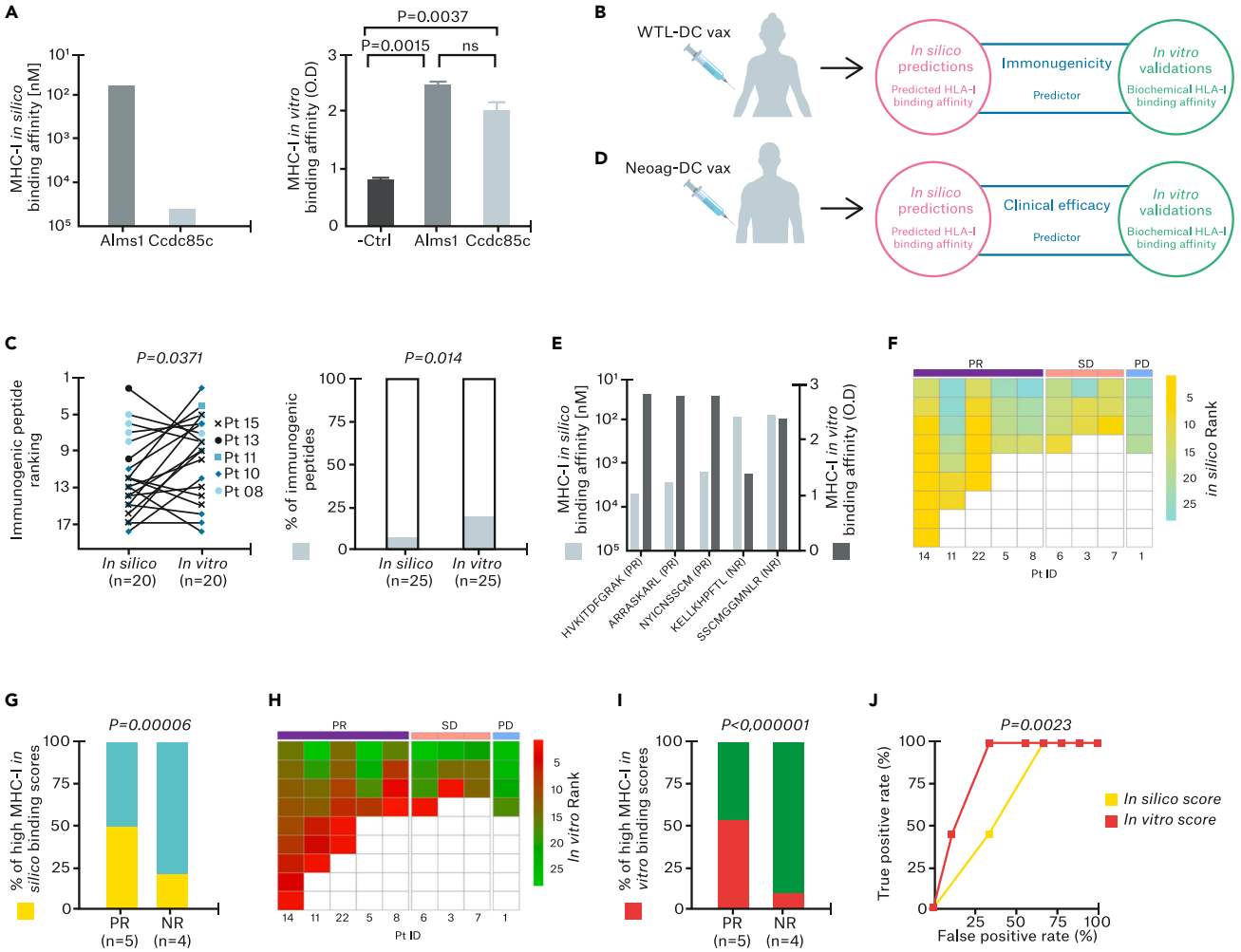

Figure 2. Effective *in vitro* binding affinity scores better predict vaccines' immunogenicity and efficacy than *in silico* predictions alone in vaccinated ovarian (OC) and advanced non-small cell lung cancer (NSCLC) patients

(A) *In silico*-predicted affinity (left, as reported in Ebrahimi-Nik et al.)[15] and effective *in vitro* binding (right) of *Ccd85c*$_{\text{YIRPFETKVK}}$ neoepitope.

(B and D) Schematic representations of the assessment of the relative accuracy of *in silico* and *in vitro* readouts to predict immunogenicity (B) and clinical efficacy (D).

(C) Relative *in silico* (as reported in Tanyi et al.)[6] and *in vitro* ranking of immunogenic peptides from the five patients for which HLA alleles were available for the assay (left, Table S2). Proportion of immunogenic peptides among the top five best *in silico* (as reported in Tanyi, et al.)[6] or the top five best *in vitro* binders in n = 5 patients (right, Table S2).

(E). Representative examples of *in silico* (as reported in Li et al.)[16] and *in vitro* binding affinities of peptides used to immunize NSCLC patients (Table S3).

(F–I) Neoepitopes ranking according to their predicted *in silico* (F and G, as reported in Li et al., Table S3) or effective *in vitro* (H and I, Table S3) binding affinity to cognate HLA class-I alleles and association with clinical responses after immunization (PR: partial responders; NR: non-responders, as reported in Li et al.). (F and H) Patients are grouped according to clinical responses (PR: partial responders; SD: stable disease; PD: progressive disease, as reported in Li et al., Table S3), and cognate peptides are color-coded based on their *in silico* (F) or *in vitro* scores (H). (G and I) Fraction of peptides with high *in silico* (G) or *in vitro* scores (I) in patients with partial responses (PR) or not (NR) after immunization (as reported in Li, et al., Table S3).

(J) Prediction of clinical outcome of *in silico* (yellow curve) and *in vitro* (red curve) readouts. Statistical analyses: Fisher's exact test and Welch's t-test.

Finally, having established that the *in vitro* binding score is a better predictor of immunogenicity than the *in silico* binding score, we asked whether the same would also apply to its association with clinical efficacy. To this end, we analyzed a cohort of non-small cell lung cancer (NSCLC) patients treated with personalized neoantigen vaccines using peptides predicted *in silico* (NetMHCpan 4.0) (Figure 2D).[16] After synthesizing 44 peptides reported in the study (Table S3) and evaluating their *in vitro* binding affinity to cognate HLA-I alleles, discrepancies between *in silico* predictions and *in vitro* binding scores were observed (Figure 2E). Next, we asked which peptide selection strategy was the best correlate of vaccines' efficacy. Compared to non-responding, patients with clinical responses were characterized by neoantigens of both higher *in silico*

(Figures 2F and 2G) and *in vitro* (Figures 2H and 2I) affinities, although the significance was stronger for the former. Furthermore, the *in vitro*-based readout (*i.e.*, the presence of peptides with a high *in vitro* binding score in vaccines) was a better predictor of clinical efficacy (Figure 2J).

DISCUSSION

The development of personalized cancer vaccines is an important breakthrough in the treatment of solid tumors. It is, however, unclear not only whether it is feasible to propose synthetic vaccines based on private antigens for the many tumor types with low mutational load but also whether synthetic vaccines based on private antigens are more effective than whole-tumor-lysate vaccines. The results presented here support the use of WTL as a source of antigen for personalized vaccines, leading to tumor control. Indeed this is in line with previous clinical data from our group in ovarian cancer patients[6,7] and others such as the recent data in glioblastoma patients[17] associating clinical benefit with WTL vaccination. Despite the potential benefit that can be derived from whole-tumor-lysate vaccination strategies, they also do have their limitations as they can only be used in indications where tumor tissue is accessible and they also do contain self-antigens as well as a plethora of other factors and signaling molecules that could dampen the immune response.

As an alternative, synthetic vaccines based on neoantigen peptides derived from *in silico* predictors can be an effective form of vaccination given that one aims at selecting the right tumor-rejection neoantigens. Indeed, although several neoantigen prioritization methods have been published, the prediction of effective neoantigens is still a challenging task demonstrated by the fact that in vaccine clinical trials only a very small fraction of the predicted neoantigens have been found to be immunogenic so far.[2,18] Moreover, the TESLA initiative has recently demonstrated that on the same dataset, different discovery pipeline outputs have little overlap and little positive predictive value (<10%).[19] Our results point out that effective *in vitro* pMHC binding and peptide immunogenicity (e.g., as included in the PRIME algorithm)[14,20] are factors which can significantly further improve the selection of *in silico*-predicted tumor-rejection antigens and should be taken into consideration to increase vaccines' clinical efficacy. In line with this, previous analyses conducted by Brennick et al. in a murine colon cancer model showed that *in silico* binding prediction with NetMHC 4.0 was indeed unable to identify most of the experimentally validated tumor-rejection neoantigens, further demonstrating the limitations of current *in silico* binding-based selection strategies for neoantigen identification.[21] On the other hand, studies conducted by the same group demonstrated that, by using common *in vitro* immunogenicity assays, they failed to detect significant CD8 T cell responses to any of the top-ranked identified tumor-rejection neoantigens[15,21] unlike what we observe in this current study whereby if immunogenicity is included *a priori* as a predictor, it could result in peptides with better tumor control. Indeed, the factors mentioned above are not by any means conclusive; in fact finding the right tumor neoantigen candidate for vaccination remains a daunting task albeit the massive advancements in technologies. We highlight here two factors that could potentially increase chances in identifying relevant antigens, but it is clear that the task of predicting immunogenicity is challenging and has limitations: a) because of the smaller size of the data available to train algorithms and b) because of the multiple other factors that influence T cell recognition (e.g., co-receptors, cytokines, etc.).[20] One way to bypass or strengthen immunogenicity prediction is through experimental validation of potential epitopes in functional cell-based assays prior to peptide manufacturing, an approach which will be incorporated in some future clinical pipelines.[22] This approach however adds another logistical and cumbersome step to the vaccine-development field. This also applies to incorporating the *in vitro* pMHC binding itself; despite its promise as demonstrated in our study, its limitations lie not only within the ability of this assay to measure peptides longer than 8-9 mers but also in prolonging vaccine manufacturing time in a disease setting where time is critical.

In summary, personalized antigen-based vaccines still hold promise whether it is through whole tumor or synthetic neoantigen approaches. However, the effective selection and prioritization of neoantigens that are able to induce a strong immune response remain a substantial hurdle. Neoantigen prioritization needs further optimization which can be achieved through better *in silico* algorithms potentially combined to improved biochemical screening assays or even a broader search space or new tools for identifying mutations outside of the current scope.

Collectively, these findings provide useful insights for the expanding cancer vaccine field where future integration with standard-of-care modalities including checkpoint blockade inhibitors,[23–25] T cell therapy,[26]

and even different prime-boost approaches[27] seem to be not far-fetched and of high importance to the field.

Limitations of the study

One potential limitation of the study is that unpulsed DCs (or DCs pulsed with an irrelevant tumor lysate) was not consistently used as additional controls. We relied on previous studies from us and others demonstrating that unpulsed DCs (or DCs pulsed with irrelevant tumor lysate) did not induce any tumor control in vaccinated tumor-bearing mice as opposed to DCs pulsed with cancer-specific antigen (or cognate tumor lysate) where improved survival outcomes were observed.[28–31] Another limitation is that the immunogenicity of wild-type peptides was not assessed for each vaccine-induced neoepitope-specific T cell response. The number of human patient samples reported in Figure 2 may also be considered as a limitation, the reason being the paucity of clinical studies currently available utilizing short peptides in their vaccination strategies. Yet, conclusions from the human studies (as currently reported in Figure 2) fully support the conclusions from the mouse studies (reported in Figure 1). Another limitation is that we only re-evaluated our results in context of netMHCpan 4.1; however, there is a diversity of other tools that may have been used in this case. Indeed, more mouse studies and human cohorts are for sure required to validate our current findings.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106288.

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AUTHOR CONTRIBUTIONS

L.E.K. and A.H. conceived, designed, and supervised the study. H.F., CL-L.C., and AL.H. performed experiments. H.F., R.P, J.S., and P.G. generated, analyzed, and interpreted the data. B.J.S. and D.G. performed the *in silico* predictions. H.F., M.G., A.H., and L.E.K. wrote, reviewed, and edited the manuscript. All authors read and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Sprooten, J., Ceusters, J., Coosemans, A., Agostinis, P., De Vleeschouwer, S., Zitvogel, L., Kroemer, G., Galluzzi, L., and Garg, A.D. (2019). Trial watch: dendritic cell vaccination for cancer immunotherapy. OncoImmunology *8*, e1638212. https://doi.org/10.1080/2162402x.2019.1638212.

2. Ott, P.A., Hu, Z., Keskin, D.B., Shukla, S.A., Sun, J., Bozym, D.J., Zhang, W., Luoma, A., Giobbie-Hurder, A., Peter, L., et al. (2017). An immunogenic personal neoantigen vaccine for patients with melanoma. Nature *547*, 217–221. https://doi.org/10.1038/nature22991.

3. Sahin, U., Derhovanessian, E., Miller, M., Kloke, B.P., Simon, P., Löwer, M., Bukur, V., Tadmor, A.D., Luxemburger, U., Schrörs, B., et al. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature *547*, 222–226. https://doi.org/10.1038/nature23003.

4. Verdon, D.J., and Jenkins, M.R. (2021). Identification and targeting of mutant peptide neoantigens in cancer immunotherapy. Cancers *13*, 4245. https://doi.org/10.3390/cancers13164245.

5. Lang, F., Schrörs, B., Löwer, M., Türeci, Ö., and Sahin, U. (2022). Identification of neoantigens for individualized therapeutic cancer vaccines. Nat. Rev. Drug Discov. *21*, 261–282. https://doi.org/10.1038/s41573-021-00387-y.

6. Tanyi, J.L., Bobisse, S., Ophir, E., Tuyaerts, S., Roberti, A., Genolet, R., Baumgartner, P., Stevenson, B.J., Iseli, C., Dangaj, D., et al. (2018). Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Sci. Transl. Med. *10*, eaao5931. https://doi.org/10.1126/scitranslmed.aao5931.

7. Tanyi, J.L., Chiang, C.L.L., Chiffelle, J., Thierry, A.C., Baumgartener, P., Huber, F., Goepfert, C., Tarussio, D., Tissot, S., Torigian, D.A., et al. (2021). Personalized cancer vaccine strategy elicits polyfunctional T cells and demonstrates clinical benefits in ovarian cancer. NPJ Vaccines *6*, 36. https://doi.org/10.1038/s41541-021-00297-5.

8. Zhong, W., Myers, J.S., Wang, F., Wang, K., Lucas, J., Rosfjord, E., Lucas, J., Hooper, A.T., Yang, S., Lemon, L.A., et al. (2020). Comparison of the molecular and cellular phenotypes of common mouse syngeneic models with human tumors. BMC Genom. *21*, 2. https://doi.org/10.1186/s12864-019-6344-3.

9. Li, S., Simoni, Y., Zhuang, S., Gabel, A., Ma, S., Chee, J., Islas, L., Cessna, A., Creaney, J., Bradley, R.K., et al. (2021). Characterization of neoantigen-specific T cells in cancer resistant to immune checkpoint therapies. Proc. Natl. Acad. Sci. USA *118*. e2025570118. https://doi.org/10.1073/pnas.2025570118.

10. Reynisson, B., Alvarez, B., Paul, S., Peters, B., and Nielsen, M. (2020). NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res. *48*, 449–454. https://doi.org/10.1093/nar/gkaa379.

11. Lundegaard, C., Lamberth, K., Harndahl, M., Buus, S., Lund, O., and Nielsen, M. (2008). NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. Nucleic Acids Res. *36*, W509–W512. https://doi.org/10.1093/nar/gkn202.

12. Garboczi, D.N., Hung, D.T., and Wiley, D.C. (1992). HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in Escherichia coli and complexed with single antigenic peptides. Proc. Natl. Acad. Sci. USA *89*, 3429–3433. https://doi.org/10.1073/pnas.89.8.3429.

13. Schmidt, J., Guillaume, P., Dojcinovic, D., Karbach, J., Coukos, G., and Luescher, I. (2017). In silico and cell-based analyses reveal strong divergence between prediction and observation of T-cell-recognized tumor antigen T-cell epitopes. J. Biol. Chem. *292*, 11840–11849. https://doi.org/10.1074/jbc.M117.789511.

14. Schmidt, J., Smith, A.R., Magnin, M., Racle, J., Devlin, J.R., Bobisse, S., Cesbron, J., Bonnet, V., Carmona, S.J., Huber, F., et al. (2021). Prediction of neo-epitope immunogenicity reveals TCR recognition determinants and provides insight into immunoediting. Cell Rep. Med. *2*, 100194. https://doi.org/10.1016/j.xcrm.2021.100194.

15. Ebrahimi-Nik, H., Moussa, M., Englander, R.P., Singhaviranon, S., Michaux, J., Pak, H., Miyadera, H., Corwin, W.L., Keller, G.L.J., Hagymasi, A.T., et al. (2021). Reversion analysis reveals the in vivo immunogenicity of a poorly MHC I-binding cancer neoepitope. Nat. Commun. *12*, 6423. https://doi.org/10.1038/s41467-021-26646-5.

16. Li, F., Deng, L., Jackson, K.R., Talukder, A.H., Katailiha, A.S., Bradley, S.D., Zou, Q., Chen, C., Huo, C., Chiu, Y., et al. (2021). Neoantigen vaccination induces clinical and immunologic responses in non-small cell lung cancer patients harboring EGFR mutations. J. Immunother. Cancer *9*, e002531. https://doi.org/10.1136/jitc-2021-002531.

17. Liau, L.M., Ashkan, K., Brem, S., Campian, J.L., Trusheim, J.E., Iwamoto, F.M., Tran, D.D., Ansstas, G., Cobbs, C.S., Heth, J.A., et al. (2023). Association of autologous tumor lysate-loaded dendritic cell vaccination with extension of survival among patients with newly diagnosed and recurrent glioblastoma: a Phase 3 prospective externally controlled cohort trial. JAMA Oncol. *9*, 112–121. https://doi.org/10.1001/jamaoncol.2022.5370.

18. Hilf, N., Kuttruff-Coqui, S., Frenzel, K., Bukur, V., Stevanović, S., Gouttefangeas, C., Platten, M., Tabatabai, G., Dutoit, V., van der Burg, S.H., et al. (2019). Publisher Correction: actively personalized vaccination trial for newly diagnosed glioblastoma. Nature *566*, E13. https://doi.org/10.1038/s41586-019-0959-z.

19. Wells, D.K., van Buuren, M.M., Dang, K.K., Hubbard-Lucey, V.M., Sheehan, K.C.F., Campbell, K.M., Lamb, A., Ward, J.P., Sidney, J., Blazquez, A.B., et al. (2020). Key parameters of tumor epitope immunogenicity revealed through a consortium approach improve neoantigen prediction. Cell *183*, 818–834.e13. https://doi.org/10.1016/j.cell.2020.09.015.

20. Gfeller, D., Schmidt, J., Croce, G., Guillaume, P., Bobisse, S., Genolet, R., Queiroz, L., Cesbron, J., Racle, J., and Harari, A. (2023). Improved predictions of antigen presentation and TCR recognition with MixMHCpred2.2 and PRIME2.0 reveal potent SARS-CoV-2 CD8(+) T-cell epitopes. Cell Syst. *14*, 72–83.e5. https://doi.org/10.1016/j.cels.2022.12.002.

21. Brennick, C.A., George, M.M., Moussa, M.M., Hagymasi, A.T., Seesi, S.A., Shcheglova, T.V., Englander, R.P., Keller, G.L., Balsbaugh, J.L., Baker, B.M., et al. (2021). An unbiased approach to defining bona fide cancer neoepitopes that elicit immune-mediated cancer rejection. J. Clin. Invest. *131*, e142823. https://doi.org/10.1172/JCI142823.

22. Sarivalasis, A., Boudousquié, C., Balint, K., Stevenson, B.J., Gannon, P.O., Iancu, E.M., Rossier, L., Martin Lluesma, S., Mathevet, P., Sempoux, C., et al. (2019). A Phase I/II trial comparing autologous dendritic cell vaccine pulsed either with personalized peptides (PEP-DC) or with tumor lysate (OC-DC) in patients with advanced high-grade ovarian serous carcinoma. J. Transl. Med. *17*, 391. https://doi.org/10.1186/s12967-019-02133-w.

23. Bjoern, J., Iversen, T.Z., Nitschke, N.J., Andersen, M.H., and Svane, I.M. (2016). Safety, immune and clinical responses in metastatic melanoma patients vaccinated with a long peptide derived from indoleamine 2,3-dioxygenase in combination with ipilimumab. Cytotherapy *18*, 1043–1055. https://doi.org/10.1016/j.jcyt.2016.05.010.

24. Wilgenhof, S., Corthals, J., Heirman, C., van Baren, N., Lucas, S., Kvistborg, P., Thielemans, K., and Neyns, B. (2016). Phase II study of autologous monocyte-derived mRNA electroporated dendritic cells (TriMixDC-MEL) plus ipilimumab in patients with pretreated advanced melanoma. J. Clin. Oncol. *34*, 1330–1338. https://doi.org/10.1200/jco.2015.63.4121.

25. Hodi, F.S., Mihm, M.C., Soiffer, R.J., Haluska, F.G., Butler, M., Seiden, M.V., Davis, T., Henry-Spires, R., MacRae, S., Willman, A., et al. (2003). Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc. Natl. Acad. Sci. USA *100*, 4712–4717. https://doi.org/10.1073/pnas.0830997100.

26. Reinhard, K., Rengstl, B., Oehm, P., Michel, K., Billmeier, A., Hayduk, N., Klein, O., Kuna, K., Ouchan, Y., Wöll, S., et al. (2020). An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science *367*, 446–453. https://doi.org/10.1126/science.aay5967.

27. Harari, A., Graciotti, M., Bassani-Sternberg, M., and Kandalaft, L.E. (2020). Antitumour dendritic cell vaccination in a priming and boosting approach. Nat. Rev. Drug Discov. *19*, 635–652. https://doi.org/10.1038/s41573-020-0074-8.

28. Fields, R.C., Shimizu, K., and Mulé, J.J. (1998). Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses in vitro and in vivo. Proc. Natl. Acad. Sci. USA *95*, 9482–9487. https://doi.org/10.1073/pnas.95.16.9482.

29. Zarnani, A.H., Torabi-Rahvar, M., Bozorgmehr, M., Zareie, M., and Mojtabavi, N. (2015). Improved efficacy of a dendritic cell-based vaccine against a murine model of colon cancer: the helper protein effect. Cancer Res. Treat. *47*, 518–526. https://doi.org/10.4143/crt.2013.241.

30. Chiang, C.L.L., Kandalaft, L.E., Tanyi, J., Hagemann, A.R., Motz, G.T., Svoronos, N., Montone, K., Mantia-Smaldone, G.M., Smith, L., Nisenbaum, H.L., et al. (2013). A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. Clin. Cancer Res. *19*, 4801–4815. https://doi.org/10.1158/1078-0432.ccr-13-1185.

31. Gatza, E., and Okada, C.Y. (2002). Tumor cell lysate-pulsed dendritic cells are more effective than TCR Id protein vaccines for active immunotherapy of T cell lymphoma. J. Immunol. *169*, 5227–5235. https://doi.org/10.4049/jimmunol.169.9.5227.

32. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics *25*, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

33. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. *20*, 1297–1303. https://doi.org/10.1101/gr.107524.110.

34. Holt, K.E., Teo, Y.Y., Li, H., Nair, S., Dougan, G., Wain, J., and Parkhill, J. (2009). Detecting SNPs and estimating allele frequencies in clonal bacterial populations by sequencing pooled DNA. Bioinformatics *25*, 2074–2075. https://doi.org/10.1093/bioinformatics/btp344.

35. Nielsen, M., and Andreatta, M. (2016). NetMHCpan-3.0; improved prediction of binding to MHC class I molecules integrating information from multiple receptor and peptide length datasets. Genome Med. *8*, 33. https://doi.org/10.1186/s13073-016-0288-x.

36. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics *29*, 15–21. https://doi.org/10.1093/bioinformatics/bts635.

37. Abrams, J.A., and Chak, A. (2014). Applying big GWAS data to clarify the role of obesity in Barrett's esophagus and esophageal adenocarcinoma. J. Natl. Cancer Inst. *106*, dju299. https://doi.org/10.1093/jnci/dju299.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Synthetic peptides	Peptide and Tetramer Core Facility (University of Lausanne, Switzerland)	Customized synthesis
Biotinylated MHC heavy and light chains	Peptide and Tetramer Core Facility (University of Lausanne, Switzerland)	In house production
GM-CSF	Peprotech	AF-315-03-1MG
IL-2	Peprotech	212-12-20
IL-4	Peprotech	AF-214-14-1MG
IFN-γ	Peprotech	AF- 315-05-1MG
LPS	Invivogen	TLRL-3PELPS
DMEM	Gibco	10569-010
ITS	Gibco	41400-045
Penicillin/Streptomicin	BioConcept	4-01F00-H
FBS	Gibco	10437-028
Critical commercial assays		
RNeasy Micro Kit	Qiagen	74004
DNeasy blood and tissue kit	Qiagen	69504
SureSelect All Exon capture kit	Agilent	5191-7407
$CD8a^+$ T Cell Isolation Kit	Miltenyi	130-104-075
pre-coated IFN-γ plate	Mabtech	3321-4HST-10
Deposited data		
scRNAseq Data	This paper	ENA: PRJEB55702
Experimental models: Cell lines		
Mouse: LLC1 cells	ATCC	CRL-1642
Mouse: B16F10 cells	ATCC	CRL-6475
Mouse: ID8 cells	Prof. Iain A. McNeish lab	
Experimental models: Organisms/strains		
Mouse: C57BL/6	Envigo	C57BL/6JOLAHSD
Software and algorithms		
Burrows-Wheeler Aligner (BWA version 0.7.13)	Li and Durbin[32]	http://maq.sourceforge.net
Genome Analysis Toolkit (v 3.6)	McKenna et al.[33]	https://gatk.broadinstitute.org/hc/en-us
Mouse reference genome (version: m9/NCBI build 37)	Holt et al.[34]	ftp-mouse.sanger.ac.uk
netMHCpan v 3.0	Nielsen and Andreatta[35]	www.cbs.dtu.dk/services/NetMHCpan-3.0
netMHCpan v 4.1	Reynisson et al.[10]	https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1
PRIME	Schmidt et al.[14]	http://prime.gfellerlab.org/
STAR software	Dobin et al.[36]	m http://code.google.com/p/rna-star/
HTSeq	Abrams and Chak[37]	https://htseq.readthedocs.io/en/release_0.9.1/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Lana Kandalaft (lana.kandalaft@chuv.ch).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Sequencing data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB55702'' and ''Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.''

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

All *in vivo* experiments were conducted according to the Ludwig Institute for Cancer Research (LICR) animal experimentation protocols approved by the Veterinary Service of Canton Vaud, Switzerland. C57BL/6 mice (Invivogen) were housed in the animal facility of the University of Lausanne (UNIL) on a 12-h light/12-h dark diurnal cycle. Food was provided *ad libitum*. Female mice were used for all experiments.

Cell cultures

Lewis Lung Cancer 1 (LLC1, ATCC), melanoma (B16F10, ATCC) and ovarian cell line (ID8 wild-type; University of Glasgow, UK) were cultivated in DMEM (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin (BioConcept). For ID8 cells, the media was supplemented with For the ID8 we added 1% of ITS (Insulin, Transferine, Selenite, Gibco).

Animal vaccination

Eight weeks old female C57BL/6 mice were implanted subcutaneously (s.c.) with LLC1 (2×10^5 cells/mouse) or B16F10 (1×10^5 cells/mouse) cells and vaccinated intradermally (i.d.) weekly for a duration of three weeks starting three days post tumor implantation. For the OC model, mice were implanted intraperitoneally (i.p.) with ID8 (5×10^6 cells/mouse) cells expressing luciferase as a reporter gene and vaccinated i.d. weekly for a duration of four weeks starting 19 days post tumor implantation. The vaccine was administrated at 1×10^6 DC/50µl formulated in PBS.

METHOD DETAILS

Nucleic acid extraction

One microgram of DNA and 2µg of RNA were extracted in triplicates from LLC1 tumor cells line using the DNeasy blood and tissue kit (Qiagen) and the RNeasy Micro Kit (Qiagen), respectively, according to manufacturer's instructions. DNA and RNA were then subjected to quality control for their integrity using the 5200 Fragment Analyzer (Agilent). Next generation sequencing was then performed by the company Fasteris (Geneva, Switzerland) using the SureSelect All Exon capture kit (Agilent) for exome library preparation, based on the mm9/NCBI build 37 mouse assembly.

Exome sequence read alignment, variant calling and annotation

To identify mouse somatic mutations, exome sequence reads were aligned to the mm9/NCBI build 37 mouse reference assembly using the Burrows-Wheeler Aligner (BWA version 0.7.13).[32] The read alignments were converted from SAM into BAM format and PCR duplicates were flagged using MarkDuplicates, part of Picard tools (version 1.130; https://broadinstitute.github.io/picard/). Nucleotide variants were called following the Genome Analysis Toolkit best practices (GATK version 3.6).[33] GATK officially supports only human sample analysis, but a search of bioinformatics community websites[26] indicated that the GATK could also be applied to mouse by restricting the external variant reference to mouse dbSNP, and by using hard filtering on the GATK variant call set to reduce the number of false positives.[27] After recalibration of the base quality scores with GATK BaseRecalibrator, variants were called in the tumor cell line and in the germline using GATK HaplotypeCaller and variants were filtered for quality using the hard filtering parameters recommended by the GATK. The resulting variants were annotated with snpEff.[25] Variants present in

both germline and tumor cell line were defined as single nucleotide polymorphisms (SNPs) whereas variants present only in the tumor cell line were defined as somatic mutations. The mouse reference genome (version: m9/NCBI build 37) was downloaded from the Wellcome Sanger Institute FTP site (ftp-mouse.sanger.ac.uk).[34] The dbSNP reference file for use with GATK, and based on the mm9 mouse assembly, was downloaded from the same site, as two separate files (for SNPs and indels) which were subsequently combined into a single VCF (the standard 'variant call format') using Picard MergeVcfs. All files were checked for integrity after downloading by md5 checksum.

In silico peptide construction and HLA-peptide binding predictions

Based on the annotated variants predicted by exome sequencing, 9-mer and 10-mer mouse wild-type peptides and the corresponding mutant peptides containing somatic mutations at each position in the peptide were created. The netMHCpan3.0[35] program based on binding affinity (BA) score was used for binding prediction of the peptides to the mouse C57BL/6 MHC alleles H2-D^b and H2-K^b. All the 10-mers that were used also had a strong binding affinity in their 9-mer form. The peptides predicted to be good binders to each MHC allele, designated strong binder (SB) present in the top 2% by rank, were identified and ranked according to their predicted binding affinity (NetMHCpanBA score). This list was further refined by using the RNAseq data to prioritize peptides based on gene expression level. For further validation, a more recent version of netMHCpan (v4.1)[10] was run on a mix of 9- and 10-mers encompassing the identified somatic mutations. PRIME1.0[14] was run using NetMHCpan4.1 for the predictions of binding of both 9- and 10-mers to MHC H2-D^b and H2-K^b.

RNAseq analysis

Gene expression was assessed by aligning the stranded 150 base single-end sequence reads to the mm9/NCBI build 37 genome assembly using the STAR software (version 2.5.3a)[36] and obtaining read counts per gene using HTSeq (version 0.9.1)[37] with the GENCODE mouse annotation GTF (version M1; https://www.gencodegenes.org/mouse/release_M1.html). The raw read counts per gene were normalized to Reads Per Kilobase of transcript per Million mapped reads (RPKM). Only mutation candidates that were present in expressed genes were selected and further pursued.

Peptide synthesis

Peptide synthesis was performed at the Peptide and Tetramer Core Facility (PTCF, University of Lausanne, Switzerland) by conventional Solid Phase Peptide Synthesis (SPPS) via standard Fmoc/tBu chemistry, using automated peptide synthesizers. Crude products were then HPLC purified (>80% pure), verified by mass spectrometry and finally kept lyophilized at -80° until further use.

In vitro pMHC binding assay for murine and human peptides

Peptides were tested for their *in vitro* ability to bind to their respective MHC/HLA molecules by a peptide-driven refolding assay.[12] Briefly, refolding was performed using biotinylated HLA heavy and light chains (PTCF) at 4°C for 72 hours in the presence of 10 μM of individual synthetic peptide. Readout was performed by ELISA using streptavidin-alkaline phosphatase. The baseline condition did not contain any peptide. For *in vivo* mouse vaccination, only peptides with binding affinity above the baseline were selected for further analysis. As a positive control for mouse H2-Kb and H2-Db, the $Ova_{257\text{-}264}$ SIINFEKL and $Gp100_{25\text{-}33}$ KVPRNQDWL peptides (PTCF) were used, respectively. For mouse H2-Kd, the *Alms1*LYLDSKSDTTV peptide was used. For the validation of human neoantigens, peptides were used as positive controls depending on the HLA haplotype (Table S4).

Vaccine design

Bone marrow-derived DCs (BMDCs) were generated by differentiating BM precursor cells from syngeneic C57BL/6 mice with 1000IU/ml GM-CSF (Peprotech) and 100IU/ml IL-4 (Peprotech) for 5 days. At Day 6, to generate the whole tumor lysate vaccine (WTL-DC Vax), BMDCs were pulsed with HOCl-oxidized B16F10 or LLC1 or ID8 tumor lysate prepared as previously described[30] at a 1:1 cell ratio for 16 hours. BMDCs were matured with 120 U/mL lipopolysaccharides (LPS) (Invivogen) and 4000U/mL interferon-gamma (IFN-γ) (Peprotech) for 16 hours. Alternatively, for neoantigen vaccination, synthetic peptides (PTCF) harboring LLC1 neoantigens (1 μg/mL) were incubated with matured BMDCs for 2 hours on the last day of cell culture (day 8), then pulsed cells were directly used for vaccination.

Assessment of tumor growth

B16F10 and LLC1 tumor burden was monitored with a digital caliper. Mice were euthanized when tumor burden reached $1cm^3$ or at the set endpoint (on day 21 post tumor implantation) for immune analysis. Mice harboring the ID8 cancer cells were monitored following animal weight (measured every 2 days until body weight exceeded >20% or when they became distressed and moribund); and bioluminescence quantification (Xenogen IVIS lumina II, PerkinElmer, Switzerland) of luciferase activity (reported as photons/sec). Bioluminescence imaging started 19 days post-tumor implantation and before the first treatment, then it was performed once every 2 weeks thereafter before the onset of ascites formation in PBS-treated control mice.

Vaccine immunogenicity

Fresh splenocytes were isolated from vaccinated mice and incubated at a cell density of 2×10^6 cells/ml for 1 week for *in vitro* stimulation with peptides at 1μg/ml/peptide, supplemented with 50U/ml IL-2 (Peprotech) every 2 days. At day 7 post- stimulation, cells were harvested and $CD8^+$ T cells were isolated using the $CD8a^+$ T Cell Isolation Kit (Miltenyi) for subsequent ELISpot assay. To this end, 2×10^5 $CD8^+$ T cells were co-cultured with 2×10^4 irradiated wild-type splenocytes pulsed with individual peptides (1μg/ml) in pre-coated IFN-γ plates (Mabtech) between 18 to 24 hours at 37°C. After incubation, IFN-γ production was detected according to manufacturer's protocol.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using the GraphPad Prism v9.1.0 (San Diego, CA, USA). Differences between averages of variables were compared using multiple unpaired t-test at the endpoint (day 21 post-tumor implantation) or One-Way ANOVA for variables with normal distribution or by using Welch t-test or Mann-Whitney nonparametric test for non-normal variables. Percentages between groups were compared using the Fisher's exact test. Receiver operating characteristic curves (ROC) were plotted for the human dataset to analyze the classification performance of the *in vitro* and *in silico* methods. ROC curves were compared using Welch's t-test. Correction for multiple comparisons was applied when necessary using the Holm-Sidak test recommended by the software. Differences between groups with p-values ≤ 0.05 (5% FDR) were considered statistically significant.