

## Research Perspective

# Leveraging a powerful allogeneic dendritic cell line towards neoantigen-based cancer vaccines

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## ABSTRACT

**In recent years, immunotherapy has finally found its place in the anti-cancer therapeutic arsenal, even becoming standard of care as first line treatment for metastatic forms. The clinical benefit provided by checkpoint blockers such as anti-PD-1/PD-L1 in many cancers revolutionized the field. However, too many patients remain refractory to these treatments due to weak baseline anti-cancer immunity. There is therefore a need to boost the frequency and function of patients' cytotoxic CD8+ cellular effectors by targeting immunogenic and tumor-restricted antigens, such as neoantigens using an efficient vaccination platform. Dendritic cells (DC) are the most powerful immune cell subset for triggering cellular immune response. However, autologous DC-based vaccines display several limitations, such as the lack of reproducibility and the limited number of cells that can be manufactured. Here we discuss the advantages of a new therapeutic vaccine based on an allogeneic Plasmacytoid DC cell line, which is easy to produce and represents a powerful platform for priming and expanding anti-neoantigen cytotoxic CD8+ T-cells.**

## INTRODUCTION

Due to the limited clinical benefit of anti-PD-1/PD-L1 therapy in many cancer indications, there is a renewed interest in therapeutic cancer vaccines to improve clinical responses. Indeed, one of the main explanations for resistance to these immune checkpoint inhibitors (ICI) is the absence of pre-existing anti-tumor immunity or the inadequacy of this immune response [1]. These therapeutic antibodies block the interaction between the inhibitory molecule PD-1 expressed on anti-tumor CD8+ T-cells and its ligand PD-L1, expressed by tumor cells. Their expected *in vivo* mechanism of action is thus to unleash the cytotoxic activity of anti-tumor effectors [2]. In addition, different reports describing the effect of the treatment of patients with ICIs in a neo-adjuvant setting strongly suggested that reinforcing the patient's own immune system led to the eradication of tumor cells, as evidenced by major or complete pathological responses [3–9]. Therefore, it is becoming increasingly clear that the

combination of ICIs with therapeutic cancer vaccines that aimed at priming or enhancing anti-tumor CD8+ T-cell effectors could increase the efficacy of each treatment used separately [10–12].

### Neoantigens as a source of tumor antigens for cancer immunotherapies

Among several potential tumor antigens that can be targeted by the immune system, neoantigens (NeoAgs) appear very attractive because they are tumor cell-specific proteins and unknown to the immune system (i.e., there is no pre-existing central immune tolerance) [13, 14]. NeoAgs were initially described as the result of non-synonymous somatic mutations [14], but they can also be derived from many other genomic abnormalities in the transcriptional and translational process leading to the synthesis of abnormal proteins [15–23]. Interestingly, the frequency of tumor somatic mutations correlates with objective response rates to ICIs in many cancers [24, 25]. Thus, these single

nucleotide variants may serve as neoantigens recognized by the immune system, leading to tumor cell death mediated by NeoAg-specific CD8<sup>+</sup> T-cells. Very recently, the number and frequency of NeoAg-specific CD8<sup>+</sup> T-cells were confirmed to be associated with the clinical outcome of adoptive cell therapy with tumor-infiltrating lymphocytes (TIL) by using an elegant approach [26]. Interestingly, it was also suggested recently that the expansion and activation of NeoAg-specific CD8<sup>+</sup> T-cells are associated with the response to ICIs in patients with metastatic urothelial carcinoma [27]. However, despite the considerable number of diverse genomic abnormalities, very few candidates are considered as “good” NeoAgs. This is due to the highly selective molecular machinery allowing the presentation of an immunogenic peptide derived from NeoAgs to the immune system through HLA class I molecules expressed by tumor cells [15, 23]. Recent significant developments in algorithms and deep machine learning have provided opportunities to identify few NeoAgs in the majority of patients, especially in cancers induced by mutagens or DNA mismatch repair [16, 17]. This is probably why therapeutic NeoAg-based cancer vaccines were first developed in melanoma [28–30]. The availability of resected tumors has led to develop vaccines also in glioblastoma, non-small-cell lung cancer (NSCLC), bladder, gastrointestinal, colorectal, urothelial, and pancreatic cancers [31–41]. All studies, except two [36, 37], have so far used private NeoAgs, i.e., identified in a single patient. Most of the clinical studies published are still in phase I or Phase I/II and despite the combination with ICIs, these vaccine approaches are not yet validated clinically.

From an immunological point of view, it is quite surprising that many studies used a vaccine regimen based on local injections of long peptides combined with adjuvants [29, 31–33, 36, 37, 41]. Indeed, these approaches were known to be rather suboptimal to prime and stimulate anti-tumor CD8<sup>+</sup> T-cells, and may even generate tolerogenic responses [42–46]. As a result, very weak NeoAg-specific CD8<sup>+</sup> T-cell responses have been obtained from patients, in contrast to NeoAg-specific CD4<sup>+</sup> T-cells which are not the main effectors of anti-tumor immune response. Indeed, except in rare cases, CD4<sup>+</sup> T-cells are not cytotoxic and cannot kill tumor cells due to the lack of expression of HLA class II molecules by tumor cells. RNA-based approaches have also been tested with no significant change in the nature and the amplitude of the anti-tumor response [30, 34]. However, Moderna and Merck have recently reported results on melanoma that will deserve attention when published. The use of adenoviral-based platform has been recently described with some interesting results in few patients [39, 40]. By contrast, the use of mature dendritic cells (DC) loaded with short peptides derived from NeoAgs has demonstrated strong expansions of cytotoxic CD8<sup>+</sup> T-cells for many NeoAgs in all melanoma patients tested [28].

## Dendritic cells are essential for the induction of anti-tumor response

Dendritic cells are perfectly equipped to process and present tumor antigen-derived peptides to naive CD8<sup>+</sup> T-cells in lymphoid organs, transforming them into effector memory cells capable of reaching to the tumor site and killing tumor cells [47, 48]. They are also very effective in reactivating circulating and tissue-resident anti-tumor memory T-cells [47]. Dendritic cells therefore appear to be of great interest for the development of a cancer vaccine based on NeoAgs, as they directly and efficiently stimulate the appropriate anti-tumor effector cells after injection, avoiding any induction of tolerance [49, 50]. However, to date, given that the main antigen-presenting platforms have used autologous DCs, they have faced major challenges: the cost of manufacturing, reproducibility, feasibility, the availability of sufficient drug product, the suboptimal efficacy of the product, the difficulty of establishing quality control of immune activity, and the heterogeneity of clinical trials since all patients were treated with a different drug product [51]. Except in prostate cancer [52] and very recently in glioblastoma [53], autologous DC-based vaccines have not yet proven their efficiency [54]. Interestingly, numerous issues can be solved using allogeneic dendritic cells [55]. Indeed, allogeneic DCs can be easily manufactured, as the cell source is independent of patients. In addition, the cell drug product is shortly available for the patients when they are enrolled and its potency to stimulate anti-tumor CD8<sup>+</sup> T-cells can be checked before infusion.

## Allogeneic plasmacytoid dendritic cells represent an efficient vaccination platform

We have developed a novel approach using an allogeneic plasmacytoid dendritic cell (PDC) line as an antigen-presentation platform showing great potency to prime and expand viral or tumor-specific CD8<sup>+</sup> T cells *in vitro* and *in vivo* in a humanized mouse model [55–65]. This off-the-shelf product is scalable, versatile, cost-effective, and guarantees the homogeneity of treatment and clinical results as the same product is used for all patients. This PDC platform, named PDC\*vac, was first evaluated with shared tumor-associated antigens in the treatment of melanoma with encouraging results [66]. This first-in-human phase I clinical trial demonstrated PDC\*vac safety and biological activity since it primed and expanded anti-tumor CD8<sup>+</sup> T-cells in patients. Moreover, we have shown the *in vitro* synergy of PDC\*vac with anti-PD-1 drug product leading to the improved expansion of anti-tumor CD8<sup>+</sup> T-cells from metastatic melanoma patients. The PDC\*vac platform adapted to lung cancer patients (PDC\*lung01 product) is currently being evaluated in the treatment of metastatic squamous and non-squamous lung cancer patients in combination with

**Table 1: Features of neoantigens**

Name	Mutated peptide	Parental Peptide	Reference
ME-1	FLDEFMEGV	FLDEFMEAV	[68]
AKAP13 Q285K	KLMNIQQKL	KLMNIQQQL	[28]

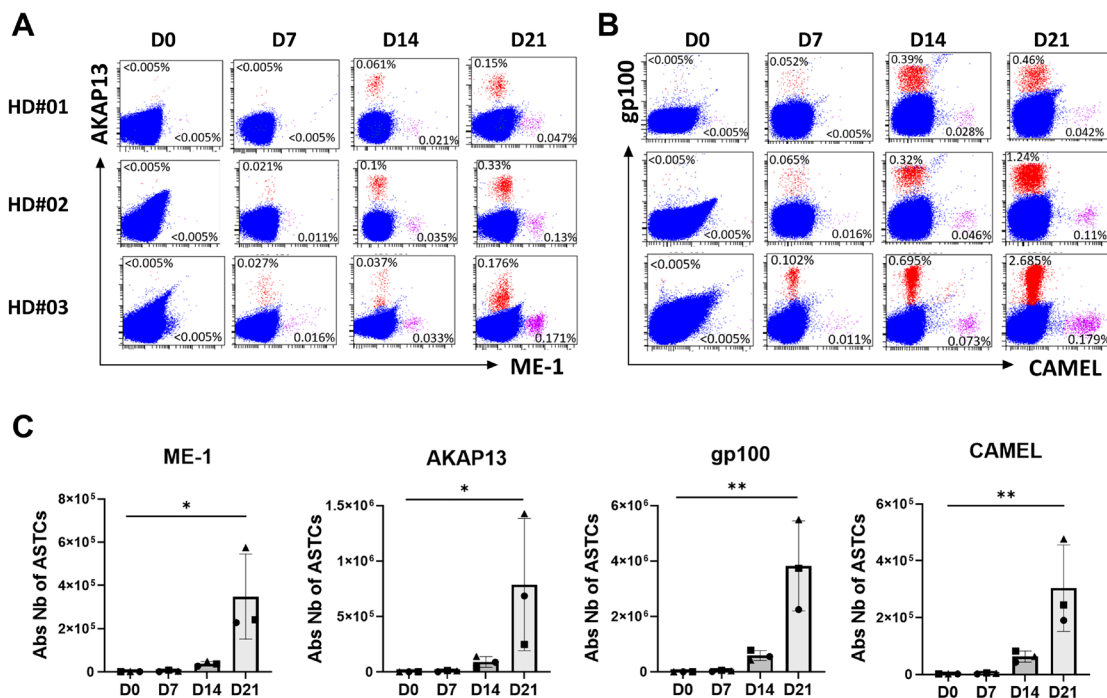
anti-PD-1 antibody (NCT03970746). The preliminary results of this phase I/II are very encouraging in terms of safety, biological, and clinical activities [67].

Given the afore-mentioned advantages of NeoAgs in vaccine approaches, we have exploited the PDC\*vac platform in order to activate NeoAg-specific immune response using the same methodology as previously described [58, 66].

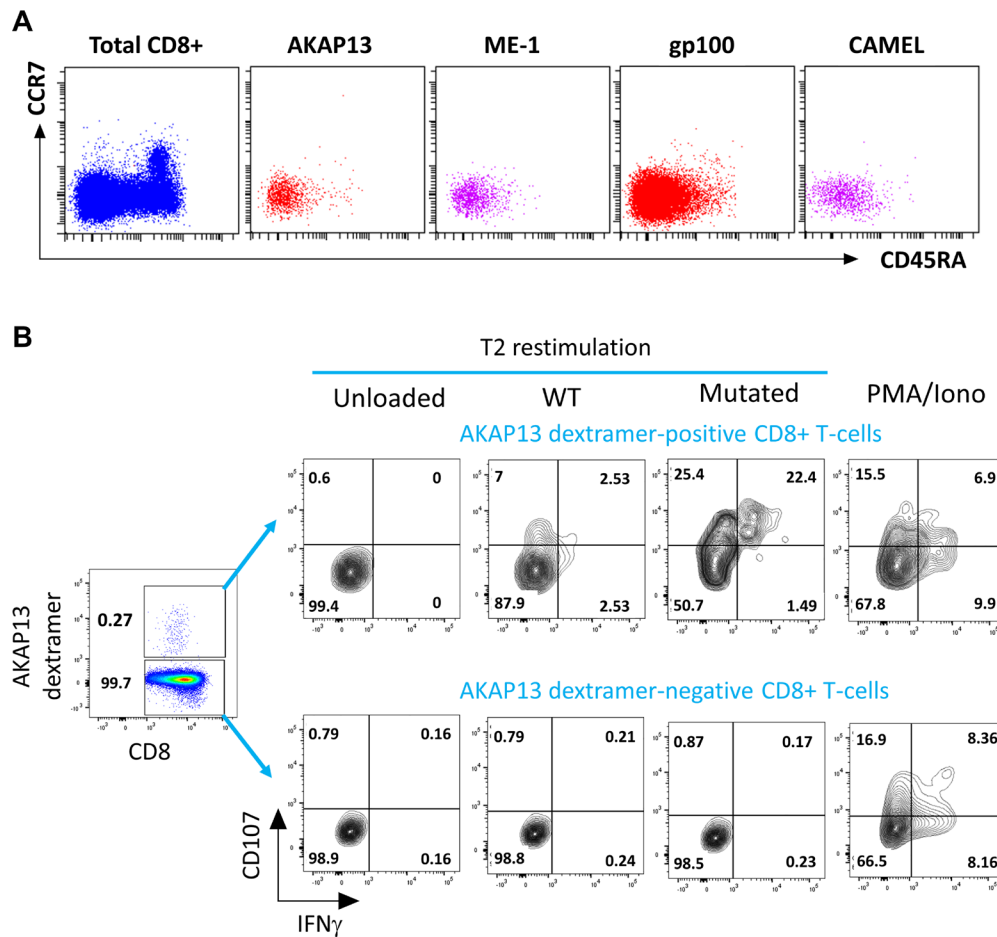
We have performed *in vitro* experiments showing that this new product named PDC\*neo can effectively prime and expand NeoAg-specific CD8<sup>+</sup> T-cells. As a proof of concept, PDC\*line cells were loaded with two NeoAgs (ME-1 and AKAP13, Table 1) already described in melanoma and lung cancer patients [28, 68] and two commonly shared tumor-associated antigens as positive controls (gp100, CAMEL). Loaded PDC\*line was then cultured with purified healthy donors' CD8<sup>+</sup> T-cells for 3 weeks before detecting specific T-cells with multimer tools (Figure 1). In such experiments, we used CD8<sup>+</sup> T-cells purified from healthy donors because they were naive,

and thus never encountered NeoAgs. As a consequence, the basal circulating precursor frequencies were expected extremely low (less or equal to 1/1,000,000 in total CD8<sup>+</sup> population). However, after weekly stimulations of these rare naive cells with PDC\*neo product, a sizeable expansion of antigen-specific CD8<sup>+</sup> T-cells was observed as soon as 7 days of co-culture, followed by a powerful expansion at day 21 (Figure 1A and 1B). Indeed, the absolute number of antigen-specific T-cells highly increases from D7 to D21 for both ME-1 and AKAP13 (Figure 1C). As expected, CAMEL- and gp100-specific T-cells were also massively primed and expanded confirming the potency of PDC\*line cells (Figure 1C).

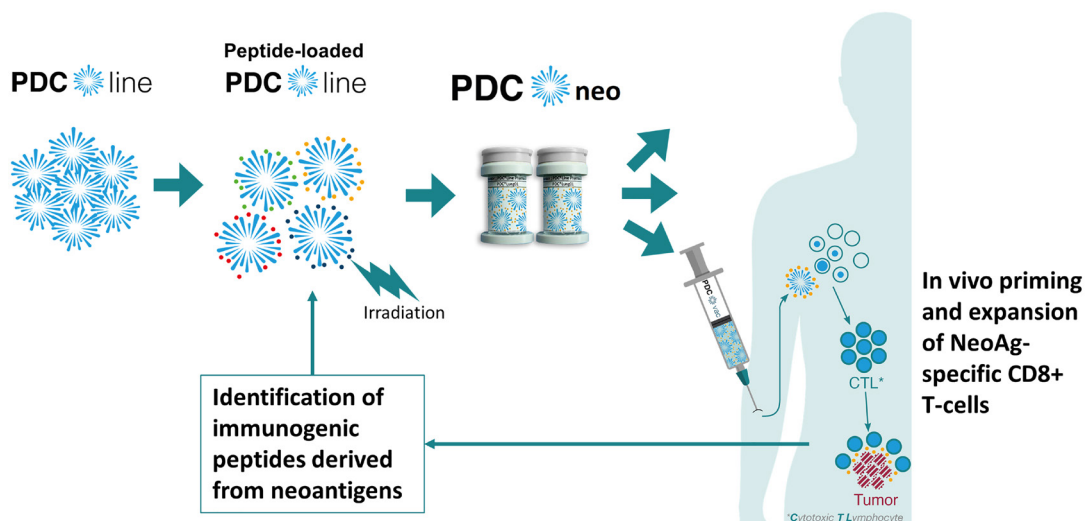
Interestingly, after 21 days of culture with PDC\*vac, all antigen-specific T-cells displayed an effector/memory phenotype (CCR7<sup>neg</sup> and CD45RA<sup>neg</sup>; Figure 2A). Moreover, the NeoAg-specific CD8<sup>+</sup> T-cells induced by PDC\*vac presented functional activity as shown by the expression of CD107 and IFN $\gamma$  upon stimulation (Figure 2B). Noteworthy, these cells were specific to



**Figure 1: Priming and expansion of NeoAg-specific T-cells by PDC\*vac.** CD8<sup>+</sup> T-cells were purified from the blood of 3 healthy donors (HD#01, HD#02, HD#03) and cocultured with peptide-loaded PDC\*line cells during 3 weeks with weekly restimulation at D7 and D14, as detailed in Lenogue et al. [58]. Antigen-specific CD8<sup>+</sup> T-cells (ASTC) were measured before (D0) and at different time points during coculture using multimer labeling. The dot plots show the proportion of CD8<sup>+</sup> T-cells specific to NeoAg (A) and to tumor-associated antigens (B) at each time point. At D0, no specific T-cells were detectable above the limit of detection of 0.005%. From D7 to D21, a continuous increase is visible for all antigens. (C) The cumulative absolute number of ASTCs is plotted at each time point, for each antigen, and for each of the 3 donors. Each symbol represents a donor: HD#01 is a filled circle, HD#02 a triangle, and HD#03 a filled square. The means of the 3 values  $\pm$  SD are shown. One-way Anova statistical analysis was performed. \* $p < 0.05$ ; \*\* $p < 0.01$ .



**Figure 2: NeoAg-specific T-cells induced by PDC\*line cells have an effector/memory phenotype, are functional and specific to the mutated antigen.** (A) Dot plots showing the CD45RA and CCR7 staining of total CD8+ T-cells and of CD8+ T cells specific to AKAP13, ME-1, gp100, and CAMEL (Donor HD#03). Naive cells are CD45RA<sup>pos</sup>CCR7<sup>pos</sup> and memory cells are CD45RA<sup>neg</sup>CCR7<sup>neg</sup>. Results are representative of one experiment. (B) Illustrative dot plots showing the expression of CD107 and IFN $\gamma$  by multimer-positive (upper line) and multimer-negative (bottom line) CD8+ T-cells from HD#02 donor upon antigenic stimulation with mutated or wild-type (WT) AKAP13 peptide. Results are representative of two experiments.



**Figure 3: The use of PDC\*vac platform to develop NeoAg-based cancer vaccines.** Peptides derived from shared or private neoantigens will be loaded on PDC\*line cells before their irradiation, packaging, and freezing. The resulting drug product will be thawed on demand and injected into patients to prime and expand NeoAg-specific T-cells *in vivo*, expecting the eradication of tumor cells.

the mutated form of the neopeptide as they did not react against the wild-type peptide.

Altogether, these data demonstrate that PDC\*vac represents an interesting tool for assessing the immunogenicity of neo-epitopes *in vitro*, as well as a powerful vaccine platform for NeoAg-based cancer vaccines. Indeed, PDC\*line is a highly potent professional antigen-presenting cell that migrates in lymph nodes and tissues (unpublished data) to directly stimulate peptide-specific CD8<sup>+</sup> T-cells. The allogeneic context may bring supplementary activation signal for the immune system. As PDC\*line cells are loaded with short peptides, there is no need of antigen transcription, translation, and processing since the peptides are directly loaded on and presented by surface HLA molecules. Finally, the direct presentation of peptides by the dendritic cells themselves avoids any unwanted tolerance induction.

## CONCLUSIONS

NeoAgs appear attractive candidates to induce specific anti-tumor responses in cancer patients, on top of classical tumor-associated antigens and in association with ICIs. A potent dendritic cell product such as PDC\*neo represents a valuable platform to develop NeoAg-based cancer vaccines (Figure 3). We strongly believe that this new delivery technology based on potent PDC\*line cells can induce a robust anti-NeoAg CD8<sup>+</sup> T-cell immune response for the benefit of patients and could reshape the landscape of NeoAg-based cancer vaccines.

## Author contributions

D. Hannani and E. Leplus performed experiments; J. Plumas supervised the study and wrote the article; K. Laulagnier, D. Hannani, and L. Chaperot reviewed the article.

## CONFLICTS OF INTEREST

Joel Plumas is Chief Scientific Officer of PDC\*line Pharma. Dalil Hannani, Estelle Leplus, and Karine Laulagnier are employees of PDC\*line Pharma.

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## REFERENCES

1. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, et al. PD-1 blockade

induces responses by inhibiting adaptive immune resistance. *Nature*. 2014; 515:568–71. <https://doi.org/10.1038/nature13954>. PMID:25428505

2. Versteven M, Van den Bergh MJM, Marcq E, Smits ELJ, Van Tendeloo VFI, Hobo W, Lion E. Dendritic Cells and Programmed Death-1 Blockade: A Joint Venture to Combat Cancer. *Front Immunol*. 2018; 9:394. <https://doi.org/10.3389/fimmu.2018.00394>. PMID:29599770
3. Topalian SL, Taube JM, Pardoll DM. Neoadjuvant checkpoint blockade for cancer immunotherapy. *Science*. 2020; 367:eaax0182. <https://doi.org/10.1126/science.aax0182>. PMID:32001626
4. Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, Wang AC, Ellingson BM, Rytlewski JA, Sanders CM, Kawaguchi ES, Du L, Li G, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat Med*. 2019; 25:477–86. <https://doi.org/10.1038/s41591-018-0337-7>. PMID:30742122
5. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, Cormier JN, Lewis C, Hwu WJ, Hanna E, Diab A, Wong MK, Royal R, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nat Med*. 2018; 24:1649–54. <https://doi.org/10.1038/s41591-018-0197-1>. PMID:30297909
6. Huang AC, Orlowski RJ, Xu X, Mick R, George SM, Yan PK, Manne S, Kraya AA, Wubbenhorst B, Dorfman L, D'Andrea K, Wenz BM, Liu S, et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. *Nat Med*. 2019; 25:454–61. <https://doi.org/10.1038/s41591-019-0357-y>. PMID:30804515
7. Blank CU, Rozeman EA, Fanchi LF, Sikorska K, van de Wiel B, Kvistborg P, Krijgsman O, van den Braber M, Philips D, Broeks A, van Thienen JV, Mallo HA, Adriaansz S, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. *Nat Med*. 2018; 24:1655–61. <https://doi.org/10.1038/s41591-018-0198-0>. PMID:30297911
8. Liu J, Blake SJ, Yong MC, Harjunpää H, Ngio SF, Takeda K, Young A, O'Donnell JS, Allen S, Smyth MJ, Teng MW. Improved Efficacy of Neoadjuvant Compared to Adjuvant Immunotherapy to Eradicate Metastatic Disease. *Cancer Discov*. 2016; 6:1382–99. <https://doi.org/10.1158/2159-8290.CD-16-0577>. PMID:27663893
9. Cascone T, William WN Jr, Weissferdt A, Leung CH, Lin HY, Pataer A, Godoy MCB, Carter BW, Federico L, Reuben A, Khan MAW, Dejima H, Francisco-Cruz A, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in operable non-small cell lung cancer: the phase 2 randomized NEOSTAR trial. *Nat Med*. 2021; 27:504–14. <https://doi.org/10.1038/s41591-020-01224-2>. PMID:33603241
10. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. *Nat Rev Cancer*. 2021; 21:360–78. <https://doi.org/10.1038/s41568-021-00346-0>. PMID:33907315

11. Garg AD, Coulie PG, Van den Eynde BJ, Agostinis P. Integrating Next-Generation Dendritic Cell Vaccines into the Current Cancer Immunotherapy Landscape. *Trends Immunol.* 2017; 38:577–93. <https://doi.org/10.1016/j.it.2017.05.006>. PMID:28610825
12. Dillman RO. Is there a role for therapeutic cancer vaccines in the age of checkpoint inhibitors? *Hum Vaccin Immunother.* 2017; 13:528–32. <https://doi.org/10.1080/21645515.2016.1244149>. PMID:27808593
13. Gilboa E. The makings of a tumor rejection antigen. *Immunity.* 1999; 11:263–70. [https://doi.org/10.1016/s1074-7613\(00\)80101-6](https://doi.org/10.1016/s1074-7613(00)80101-6). PMID:10514004
14. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science.* 2015; 348:69–74. <https://doi.org/10.1126/science.aaa4971>. PMID:25838375
15. Smith CC, Selitsky SR, Chai S, Armistead PM, Vincent BG, Serody JS. Alternative tumour-specific antigens. *Nat Rev Cancer.* 2019; 19:465–78. <https://doi.org/10.1038/s41568-019-0162-4>. PMID:31278396
16. De Mattos-Arruda L, Vazquez M, Finotello F, Lepore R, Porta E, Hundal J, Amengual-Rigo P, Ng CKY, Valencia A, Carrillo J, Chan TA, Guallar V, McGranahan N, et al. Neoantigen prediction and computational perspectives towards clinical benefit: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol.* 2020; 31:978–90. <https://doi.org/10.1016/j.annonc.2020.05.008>. PMID:32610166
17. Lybaert L, Lefever S, Fant B, Smits E, De Geest B, Breckpot K, Dirix L, Feldman SA, van Criekinge W, Thielemans K, van der Burg SH, Ott PA, Bogaert C. Challenges in neoantigen-directed therapeutics. *Cancer Cell.* 2023; 41:15–40. <https://doi.org/10.1016/j.ccell.2022.10.013>. PMID:36368320
18. Koster J, Plasterk RHA. A library of Neo Open Reading Frame peptides (NOPs) as a sustainable resource of common neoantigens in up to 50% of cancer patients. *Sci Rep.* 2019; 9:6577. <https://doi.org/10.1038/s41598-019-42729-2>. PMID:31036835
19. Roudko V, Bozkus CC, Orfanelli T, McClain CB, Carr C, O'Donnell T, Chakraborty L, Samstein R, Huang KL, Blank SV, Greenbaum B, Bhardwaj N. Shared Immunogenic Poly-Epitope Frameshift Mutations in Microsatellite Unstable Tumors. *Cell.* 2020; 183:1634–49.e17. <https://doi.org/10.1016/j.cell.2020.11.004>. PMID:33259803
20. Zhang M, Fritsche J, Roszik J, Williams LJ, Peng X, Chiu Y, Tsou CC, Hoffgaard F, Goldfinger V, Schoor O, Talukder A, Forget MA, Haymaker C, et al. RNA editing derived epitopes function as cancer antigens to elicit immune responses. *Nat Commun.* 2018; 9:3919. <https://doi.org/10.1038/s41467-018-06405-9>. PMID:30254248
21. Hoyos LE, Abdel-Wahab O. Cancer-Specific Splicing Changes and the Potential for Splicing-Derived Neoantigens. *Cancer Cell.* 2018; 34:181–83. <https://doi.org/10.1016/j.ccell.2018.07.008>. PMID:30107172
22. Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, Chambers T, Salgado R, Savas P, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol.* 2017; 18:1009–21. [https://doi.org/10.1016/S1470-2045\(17\)30516-8](https://doi.org/10.1016/S1470-2045(17)30516-8). PMID:28694034
23. Laumont CM, Perreault C. Exploiting non-canonical translation to identify new targets for T cell-based cancer immunotherapy. *Cell Mol Life Sci.* 2018; 75:607–21. <https://doi.org/10.1007/s00018-017-2628-4>. PMID:28823056
24. Yarchoan M, Johnson BA 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. *Nat Rev Cancer.* 2017; 17:209–22. <https://doi.org/10.1038/nrc.2016.154>. PMID:28233802
25. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015; 348:124–28. <https://doi.org/10.1126/science.aaa1348>. PMID:25765070
26. Kristensen NP, Heeke C, Tvingsholm SA, Borch A, Draghi A, Crowther MD, Carri I, Munk KK, Holm JS, Bjerregaard AM, Bentzen AK, Marquard AM, Szallasi Z, et al. Neoantigen-reactive CD8+ T cells affect clinical outcome of adoptive cell therapy with tumor-infiltrating lymphocytes in melanoma. *J Clin Invest.* 2022; 132:e150535. <https://doi.org/10.1172/JCI150535>. PMID:34813506
27. Holm JS, Funt SA, Borch A, Munk KK, Bjerregaard AM, Reading JL, Maher C, Regazzi A, Wong P, Al-Ahmadie H, Iyer G, Tamhane T, Bentzen AK, et al. Neoantigen-specific CD8 T cell responses in the peripheral blood following PD-L1 blockade might predict therapy outcome in metastatic urothelial carcinoma. *Nat Commun.* 2022; 13:1935. <https://doi.org/10.1038/s41467-022-29342-0>. PMID:35410325
28. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science.* 2015; 348:803–8. <https://doi.org/10.1126/science.aaa3828>. PMID:25837513
29. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature.* 2017; 547:217–21. <https://doi.org/10.1038/nature22991>. PMID:28678778
30. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Löwer M, Bukur V, Tadmor AD, Luxemburger U, Schrörs B, Omokoko T, Vormehr M, Albrecht C, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature.* 2017; 547:222–26. <https://doi.org/10.1038/nature23003>. PMID:28678784

31. Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanović S, Gouttefangeas C, Platten M, Tabatabai G, Dutoit V, van der Burg SH, Thor Straten P, Martínez-Ricarte F, Ponsati B, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature*. 2019; 565:240–45. <https://doi.org/10.1038/s41586-018-0810-y>. PMID:30568303
32. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gjini E, Shukla SA, Hu Z, Li L, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature*. 2019; 565:234–39. <https://doi.org/10.1038/s41586-018-0792-9>. PMID:30568305
33. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, Friedlander T, Bushway ME, Balogh KN, et al. A Phase Ib Trial of Personalized Neoantigen Therapy Plus Anti-PD-1 in Patients with Advanced Melanoma, Non-small Cell Lung Cancer, or Bladder Cancer. *Cell*. 2020; 183:347–62.e24. <https://doi.org/10.1016/j.cell.2020.08.053>. PMID:33064988
34. Cafri G, Gartner JJ, Zaks T, Hopson K, Levin N, Paria BC, Parkhurst MR, Yossef R, Lowery FJ, Jafferji MS, Prickett TD, Goff SL, McGowan CT, et al. mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. *J Clin Invest*. 2020; 130:5976–88. <https://doi.org/10.1172/JCI134915>. PMID:33016924
35. Bassani-Sternberg M, Digkila A, Huber F, Wagner D, Sempoux C, Stevenson BJ, Thierry AC, Michaux J, Pak H, Racle J, Boudousquie C, Balint K, Coukos G, et al. A Phase Ib Study of the Combination of Personalized Autologous Dendritic Cell Vaccine, Aspirin, and Standard of Care Adjuvant Chemotherapy Followed by Nivolumab for Resected Pancreatic Adenocarcinoma-A Proof of Antigen Discovery Feasibility in Three Patients. *Front Immunol*. 2019; 10:1832. <https://doi.org/10.3389/fimmu.2019.01832>. PMID:31440238
36. Mueller S, Taitt JM, Villanueva-Meyer JE, Bonner ER, Nejo T, Lulla RR, Goldman S, Banerjee A, Chi SN, Whipple NS, Crawford JR, Gauvain K, et al. Mass cytometry detects H3.3K27M-specific vaccine responses in diffuse midline glioma. *J Clin Invest*. 2020; 130:6325–37. <https://doi.org/10.1172/JCI140378>. PMID:32817593
37. Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, Sahm F, Sanghvi K, Tan CL, Poschke I, Green E, Justesen S, Behrens GA, et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature*. 2021; 592:463–68. <https://doi.org/10.1038/s41586-021-03363-z>. PMID:33762734
38. Cai Z, Su X, Qiu L, Li Z, Li X, Dong X, Wei F, Zhou Y, Luo L, Chen G, Chen H, Wang Y, Zeng Y, Liu X. Personalized neoantigen vaccine prevents postoperative recurrence in hepatocellular carcinoma patients with vascular invasion. *Mol Cancer*. 2021; 20:164. <https://doi.org/10.1186/s12943-021-01467-8>. PMID:34903219
39. D'Alise AM, Brasu N, De Intinis C, Leoni G, Russo V, Langone F, Baev D, Micarelli E, Petiti L, Picelli S, Fakih M, Le DT, Overman MJ, et al. Adenoviral-based vaccine promotes neoantigen-specific CD8(+) T cell stemness and tumor rejection. *Sci Transl Med*. 2022; 14:eabo7604. <https://doi.org/10.1126/scitranslmed.abo7604>. PMID:35947675
40. Palmer CD, Rappaport AR, Davis MJ, Hart MG, Scallan CD, Hong SJ, Gitlin L, Kraemer LD, Kounlavouth S, Yang A, Smith L, Schenk D, Skoberne M, et al. Individualized, heterologous chimpanzee adenovirus and self-amplifying mRNA neoantigen vaccine for advanced metastatic solid tumors: phase 1 trial interim results. *Nat Med*. 2022; 28:1619–29. <https://doi.org/10.1038/s41591-022-01937-6>. PMID:35970920
41. Awad MM, Govindan R, Balogh KN, Spigel DR, Garon EB, Bushway ME, Poran A, Sheen JH, Kohler V, Esaulova E, Srouji J, Ramesh S, Vyasamneni R, et al. Personalized neoantigen vaccine NEO-PV-01 with chemotherapy and anti-PD-1 as first-line treatment for non-squamous non-small cell lung cancer. *Cancer Cell*. 2022; 40:1010–26.e11. <https://doi.org/10.1016/j.ccell.2022.08.003>. PMID:36027916
42. Vansteenkiste JF, Cho BC, Vanakesa T, De Pas T, Zielinski M, Kim MS, Jassem J, Yoshimura M, Dahabreh J, Nakayama H, Havel L, Kondo H, Mitsudomi T, et al. Efficacy of the MAGE-A3 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive non-small-cell lung cancer (MAGRIT): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2016; 17:822–35. [https://doi.org/10.1016/S1470-2045\(16\)00099-1](https://doi.org/10.1016/S1470-2045(16)00099-1). PMID:27132212
43. Butts C, Socinski MA, Mitchell PL, Thatcher N, Havel L, Krzakowski M, Nawrocki S, Ciuleanu TE, Bosquée L, Trigo JM, Spira A, Tremblay L, Nyman J, et al, and START trial team. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2014; 15:59–68. [https://doi.org/10.1016/S1470-2045\(13\)70510-2](https://doi.org/10.1016/S1470-2045(13)70510-2). PMID:24331154
44. Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, Cunningham D, Falk S, Wadd N, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): an open-label, randomised, phase 3 trial. *Lancet Oncol*. 2014; 15:829–40. [https://doi.org/10.1016/S1470-2045\(14\)70236-0](https://doi.org/10.1016/S1470-2045(14)70236-0). PMID:24954781
45. Hailemichael Y, Dai Z, Jaffarizad N, Ye Y, Medina MA, Huang XF, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W, Liu C, Lou Y, Wang Z, et al. Persistent antigen at vaccination sites induces tumor-specific CD8<sup>+</sup> T cell sequestration, dysfunction and deletion. *Nat Med*. 2013; 19:465–72. <https://doi.org/10.1038/nm.3105>. PMID:23455713
46. Narita M, Watanabe N, Yamahira A, Hashimoto S, Tochiki N, Saitoh A, Kaji M, Nakamura T, Furukawa T, Toba K, Fuse I, Aizawa Y, Takahashi M. A leukemic plasmacytoid dendritic cell line, PMDC05, with the ability to secrete IFN- $\alpha$  by stimulation via Toll-like receptors

- and present antigens to naïve T cells. *Leuk Res.* 2009; 33:1224–32. <https://doi.org/10.1016/j.leukres.2009.03.047>. PMID:19443030
47. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000; 18:767–811. <https://doi.org/10.1146/annurev.immunol.18.1.767>. PMID:10837075
  48. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol.* 2012; 30:1–22. <https://doi.org/10.1146/annurev-immunol-100311-102839>. PMID:22136168
  49. Stevens D, Ingels J, Van Lint S, Vandekerckhove B, Vermaelen K. Dendritic Cell-Based Immunotherapy in Lung Cancer. *Front Immunol.* 2020; 11:620374. <https://doi.org/10.3389/fimmu.2020.620374>. PMID:33679709
  50. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, Guo C, Wu X, Li Y, Li X, Li G, Xiong W, Zeng Z. Neoantigen vaccine: an emerging tumor immunotherapy. *Mol Cancer.* 2019; 18:128. <https://doi.org/10.1186/s12943-019-1055-6>. PMID:31443694
  51. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol.* 2020; 20:7–24. <https://doi.org/10.1038/s41577-019-0210-z>. PMID:31467405
  52. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF, and IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med.* 2010; 363:411–22. <https://doi.org/10.1056/NEJMoa1001294>. PMID:20818862
  53. Liao LM, Ashkan K, Brem S, Campian JL, Trusheim JE, Iwamoto FM, Tran DD, Ansstas G, Cobbs CS, Heth JA, Salacz ME, D'Andre S, Aiken RD, et al. 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.* 2023; 9:112–21. <https://doi.org/10.1001/jamaoncol.2022.5370>. PMID:36394838
  54. Sprooten J, Ceusters J, Coosemans A, Agostinis P, De Vleeschouwer S, Zitvogel L, Kroemer G, Galluzzi L, Garg AD. Trial watch: dendritic cell vaccination for cancer immunotherapy. *Oncoimmunology.* 2019; 8:e1638212. <https://doi.org/10.1080/2162402X.2019.1638212>. PMID:31646087
  55. Plumas J. Harnessing dendritic cells for innovative therapeutic cancer vaccines. *Curr Opin Oncol.* 2022; 34:161–68. <https://doi.org/10.1097/CCO.0000000000000815>. PMID:34930882
  56. Aspod C, Charles J, Leccia MT, Laurin D, Richard MJ, Chaperot L, Plumas J. A novel cancer vaccine strategy based on HLA-A\*0201 matched allogeneic plasmacytoid dendritic cells. *PLoS One.* 2010; 5:e10458. <https://doi.org/10.1371/journal.pone.0010458>. PMID:20454561
  57. Aspod C, Leccia MT, Salameire D, Laurin D, Chaperot L, Charles J, Plumas J. HLA-A(\*)0201(+) plasmacytoid dendritic cells provide a cell-based immunotherapy for melanoma patients. *J Invest Dermatol.* 2012; 132:2395–406. <https://doi.org/10.1038/jid.2012.152>. PMID:22696054
  58. Lenogue K, Walencik A, Laulagnier K, Molens JP, Benlalam H, Dreno B, Coulie P, Pule M, Chaperot L, Plumas J. Engineering a Human Plasmacytoid Dendritic Cell-Based Vaccine to Prime and Expand Multispecific Viral and Tumor Antigen-Specific T-Cells. *Vaccines (Basel).* 2021; 9:141. <https://doi.org/10.3390/vaccines9020141>. PMID:33578850
  59. Aspod C, Laurin D, Richard MJ, Vie H, Chaperot L, Plumas J. Induction of antiviral cytotoxic T cells by plasmacytoid dendritic cells for adoptive immunotherapy of posttransplant diseases. *Am J Transplant.* 2011; 11:2613–26. <https://doi.org/10.1111/j.1600-6143.2011.03722.x>. PMID:21883919
  60. Aspod C, Leloup C, Reche S, Plumas J. pDCs efficiently process synthetic long peptides to induce functional virus- and tumour-specific T-cell responses. *Eur J Immunol.* 2014; 44:2880–92. <https://doi.org/10.1002/eji.201444588>. PMID:25043392
  61. Lui G, Manches O, Chaperot L, Ducrot T, Molens JP, Sotto JJ, Bensa JC, Plumas J. Preparation of purified lymphoma cells suitable for therapy. *Cytotherapy.* 2004; 6:235–43. <https://doi.org/10.1080/14653240410006059>. PMID:15203980
  62. Martinet J, Leroy V, Dufeu-Duchesne T, Larrat S, Richard MJ, Zoulim F, Plumas J, Aspod C. Plasmacytoid dendritic cells induce efficient stimulation of antiviral immunity in the context of chronic hepatitis B virus infection. *Hepatology.* 2012; 56:1706–18. <https://doi.org/10.1002/hep.25879>. PMID:22707082
  63. Veron P, Boutin S, Martin S, Chaperot L, Plumas J, Davoust J, Masurier C. Highly efficient transduction of human plasmacytoid dendritic cells without phenotypic and functional maturation. *J Transl Med.* 2009; 7:10. <https://doi.org/10.1186/1479-5876-7-10>. PMID:19173717
  64. Angel J, Chaperot L, Molens JP, Mezin P, Amacker M, Zurbriggen R, Grichine A, Plumas J. Virosome-mediated delivery of tumor antigen to plasmacytoid dendritic cells. *Vaccine.* 2007; 25:3913–21. <https://doi.org/10.1016/j.vaccine.2007.01.101>. PMID:17336432
  65. Manches O, Lui G, Molens JP, Sotto JJ, Chaperot L, Plumas J. Whole lymphoma B cells allow efficient cross-presentation of antigens by dendritic cells. *Cytotherapy.* 2008; 10:642–49. <https://doi.org/10.1080/14653240802317647>. PMID:18836919
  66. Charles J, Chaperot L, Hannani D, Bruder Costa J, Templier I, Trabelsi S, Gil H, Moisan A, Persoons V, Hegelhofer H, Schir E, Quesada JL, Mendoza C, et al. An innovative plasmacytoid dendritic cell line-based cancer vaccine primes and expands antitumor T-cells in melanoma

- patients in a first-in-human trial. *Oncoimmunology*. 2020; 9:1738812. <https://doi.org/10.1080/2162402X.2020.1738812>. PMID:32313721
67. Vansteenkiste JF, Demedts I, Pons-Tostivint E, Biesma B, Borm F, Colinet B, Cuppens K, Derijke S, Greillier L, Jürgens J, Moro-Sibilot D, Pérol M, Sebastian M, et al. 1176P Open-label, dose escalation, phase I/II study to assess safety, tolerability, immunogenicity and preliminary clinical activity of the therapeutic cancer vaccine PDC\*lung01 with or without anti-programmed death-1 (PD-1) treatment in patients with non-small cell lung cancer (NSCLC). *Ann Oncol*. 2022; 33:S1086. <https://doi.org/10.1016/j.annonc.2022.07.1299>.
  68. Karanikas V, Colau D, Baurain JF, Chiari R, Thonnard J, Gutierrez-Roelens I, Goffinet C, Van Schaftingen EV, Weynants P, Boon T, Coulie PG. High frequency of cytolytic T lymphocytes directed against a tumor-specific mutated antigen detectable with HLA tetramers in the blood of a lung carcinoma patient with long survival. *Cancer Res*. 2001; 61:3718–24. PMID:11325844