Tumour Neoantigens: Towards Personalized Cancer Immunotherapy

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Introduction

The dual role of the immune system in cancer development and progression is now beyond doubt; it inhibits tumour cells but at the same time sculpts their immunogenicity in a Darwinian process of selection, which in turn promotes a more aggressive tumour growth. There are three phases in this so-called "immunoediting hypothesis": elimination, equilibrium and escape. Additionally, tumours use many strategies to evade the immune system. Thus, the antitumour T cell response is only a part of the **Cancer-Immunity Cycle.**

Given this situation, it seems interesting to fight cancer by enhancing the patient's immune system and tip the balance in favour of immunity as a result. In fact, the recent clinical success of several anticancer immunotherapy approaches has provided a boost to the field.

The main purpose of this project is to review the current status and future challenges of developing neoantigen-based personalized immunotherapy.

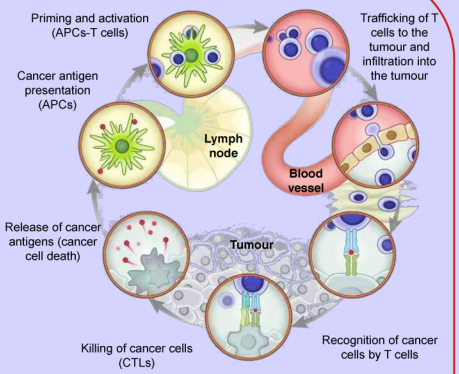


Figure 1 | The Cancer-Immunity Cycle. The generation of immunity to cancer is a cyclic process that can be divided into seven major steps, starting with the release of antigens from the cancer cell and ending with the killing of cancer cells. Abbreviations: APC, antigen presenting cells; CTLs, cytotoxic T lymphocytes. Adapted from references [1] and [2].

Tumour Neoantigens

The basis of the immune system is the recognition between self from non-self, or between "what is dangerous" from "what is not". With cancer, the fact that the targets are one's own cells makes it difficult for the immune system to distinguish between normal and cancer cells. However, in a significant minority of patients, some autologous T cell populations are able to recognize antigens present on the malignant cell's surface. One of these antigen types is called **neoantigens** and arise as a consequence of missense mutations. That is because they are tumour exclusive.

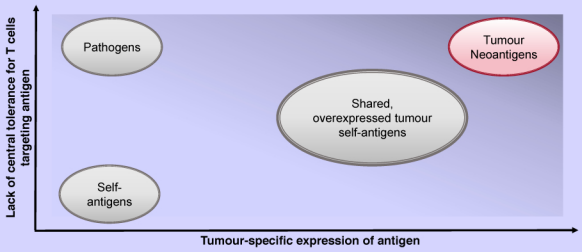


Figure 2 | Tumour neoantigens may be ideal targets for cell-based immunotherapy approaches. Tumour neoantigens (top right) are present in tumour cells but not in normal cells, so do not induce deletion of their cognate antigen-specific T cells in the thymus. In these 2 dimensions, they appear more comparable with pathogen-derived antigens (top left) than to self-antigens (bottom left). In contrast, the most commonly identified and used tumour antigens (middle) are selectively overexpressed in the tumour but can have residual expression in nontumour cells and induce central tolerance in the thymus. Adapted from reference [3].

NGS and Epitope Prediction for Assessing Neoantigen T-cell Responses

Despite current limitations of epitope prediction, next generation sequencing (NGS) has been used successfully to identify reactive neoantigen-specific T-cells in the settings of conventional cancer therapy, checkpoint blockade, vaccines and adoptive cell therapy (ACT).

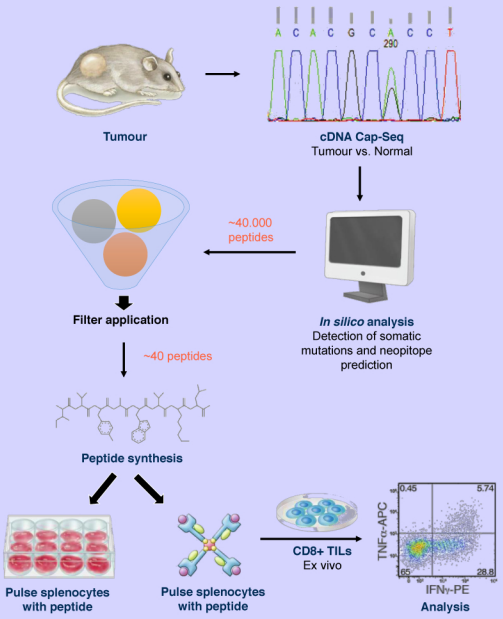


Figure 3 | Cancer exome-based identification of neoantigens. Tumour cells and normal tissue are subjected to exome-capture to identify expressed, nonsynonymous somatic mutations. Corresponding mutant epitopes are then analysed *in silico* for MHC-I binding. After applying a series of filters, the candidate peptides are synthesized and used to identify neoantigen-specific T cells in freshly explanted TILs using MHC multimer-based screens or cytokine induction by peptide stimulation. Abbreviations: MHC, major histocompatibility complex; TIL, tumour infiltrating lymphocytes Adapted from reference [4].

Neoantigens as a Biomarker for Patient Survival

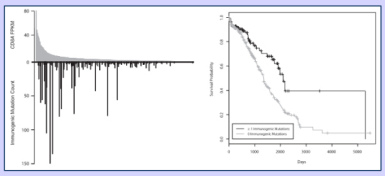


Figure 4a | The number of predicted immunogenic mutations is positively associated with CD8+ T cell infiltration (left) and patient survival (right). After analysing the potential immunogenicity of a number of missense mutations from 515 tumours, those whose immunogenicity was predicted show a strong correlation with patient survival and intratumoral CD8+ T cell expression. Reference [5].

Neoantigens and Checkpoint Blockade Therapy

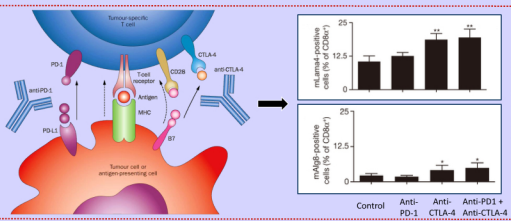


Figure 4b | Neoantigens are important for effective anti-tumour responses induced by immune checkpoint blockade. After treating tumour- bearing mice with either PD1 or CTLA4 blocking antibodies, NGS was used to identify two neoantigen-specific T cell responses in TIL, which were strongly activated by checkpoint blockade. References [6] and [7].

Neoantigens as Vaccine Targets

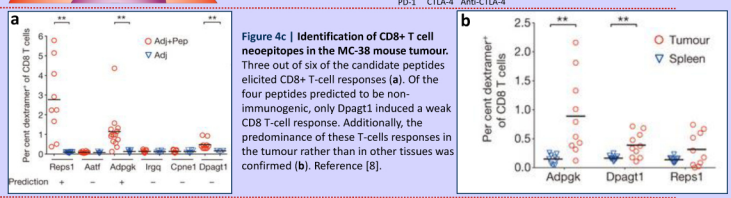


Figure 4c | Identification of CD8+ T cell neoepitopes in the MC-38 mouse tumour. Three out of six of the candidate peptides elicited CD8+ T-cell responses (**a**). Of the four peptides predicted to be non-immunogenic, only Dpagt1 induced a weak CD8 T-cell response. Additionally, the predominance of these T-cells responses in the tumour rather than in other tissues was confirmed (**b**). Reference [8].

Neoantigens and Adoptive Cell Therapy

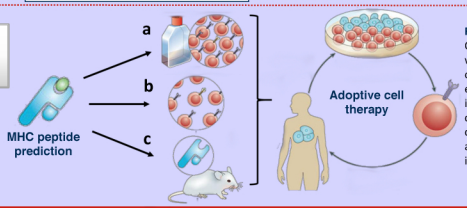


Figure 4d | Neoantigen-specific T cells in ACT. Candidate neopeptides could be used in three different ways: use tetramers to identify or sort naturally occurring T cells (**a**), culture *in vitro* with peptides to expand T cell populations derived from TIL (**b**), or immunize MHC-transgenic mice to create new TCRs and clone the TCR gene into a viral vector (**c**). In all cases, T cells that have reactivity against the tumour will be adoptively transferred into the patient to enhance his immune response. Adapted from reference [9].

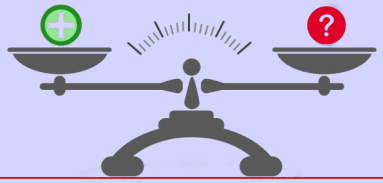
Future Challenges and Directions

Possible Clinical Applications

1. Stratify patients for checkpoint blockade according to the number of predicted neoantigens in their tumours.
2. Determine the effective dose and duration of checkpoint blockade by monitoring neoantigen-specific T cell responses over time.
3. Design personalized neoantigen-based vaccines.
4. Generate neoantigen-specific T cells for ACT.

Unanswered Questions

- How does this approach overcome tumour heterogeneity?
- What about cancers with low mutation rates?
- Can neoantigens induce tolerance?
- Are the number of neoantigen-specific T cells sufficient to deal with tumour microenvironment?
- Is this approach too impractical for widespread use?



Conclusions

The analysis of cancer genomes has revealed that tumour mutations are extremely variable among patients, even when it is the same type of cancer. There is a need for personalized cancer therapy and, in this regard, potentiation of the antitumor immune response seems to be a promising strategy.

Data supporting the critical role of neoantigens in immune control of cancer has increased in the last few years. Although it is still a young field and there is much room for improvement, what we know now for certain is:

- Neoantigens represent important targets in tumour-infiltrating lymphocyte (TIL) populations in patients benefiting from adoptive cell therapy (ACT).
- Detection of neoantigens by next generation sequencing and bioinformatics shows that processing and presentation of multiple neoantigens occurs spontaneously.
- Checkpoint blockade therapy has revealed new and amplified neoantigen-specifc responses.

Methodology

❑ Sources

Data has been obtained from research papers and reviews, as well as other specialized literature (immunology texts, dissertations, expert video conferences and monographs).

Most of the information has been collected using scientific databases, such as Pubmed, Scopus and Science Direct and applying the following selection criteria.

❑ Selection Criteria

Most Used Keywords: *neoantigen, cancer immunotherapy, vaccine, mutanome*.

Year and Journal of Publication: research has been limited mainly to the past 5 years of publication and acquired from well indexed journals, such as *Nature, Science, N Eng J Med...*

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[1] Chen S, Mellman I. Oncology Meets Immunology: the Cancer-Immunity Cycle. *Immunity* 2013; 39:1-10. [2] Cell Press Selections: Immunooncology, The Cancer Immunity Cycle Poster. [3] Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines. *Cancer Immunol Res* 2013; 1:11-15. [4] Gubin MM, Artyomov MN, Mardis ER, Schreiber RD. Tumor neoantigens: Building a Framework for Personalized Cancer Immunotherapy. *J Clin Invest* 2015; 125:3413-21. [5] Brown SD, Warren RL, Gibb EA et al. Neo-antigens Predicted by Tumor Genome Meta-analysis Correlate with Increased Patient Survival. *Genome Res* 2014;24:743-50. [6] Drake CG et al. Breathing New Life of Melanoma, Lung and Kidney Cancer. *Nat Rev Oncol* 2013. [7] Gubin MM, Zhang X, Schuster H et al. Checkpoint Blockade Cancer Immunotherapy Targets Tumour-Specific Mutant Antigens. *Nature* 2014;515:577-81. [8] Yadav M, Jhunjhunwala S, Phung QT et al. Predicting Immunogenic Tumour Mutations by Combining Mass Spectrometry and Exome Sequencing. *Nature* 2014;515:572-76. [9] Restifo NP, Dudley ME, Rosenberg SA. Adoptive Immunotherapy for Cancer: Harnessing the T Cell Response. *Nat Rev Immunol* 2012;12:269-81.