

# Development of a Potency Assay for MelCancerVac®: Dendritic Cells Pulsed with an Allogeneic Tumour Lysate



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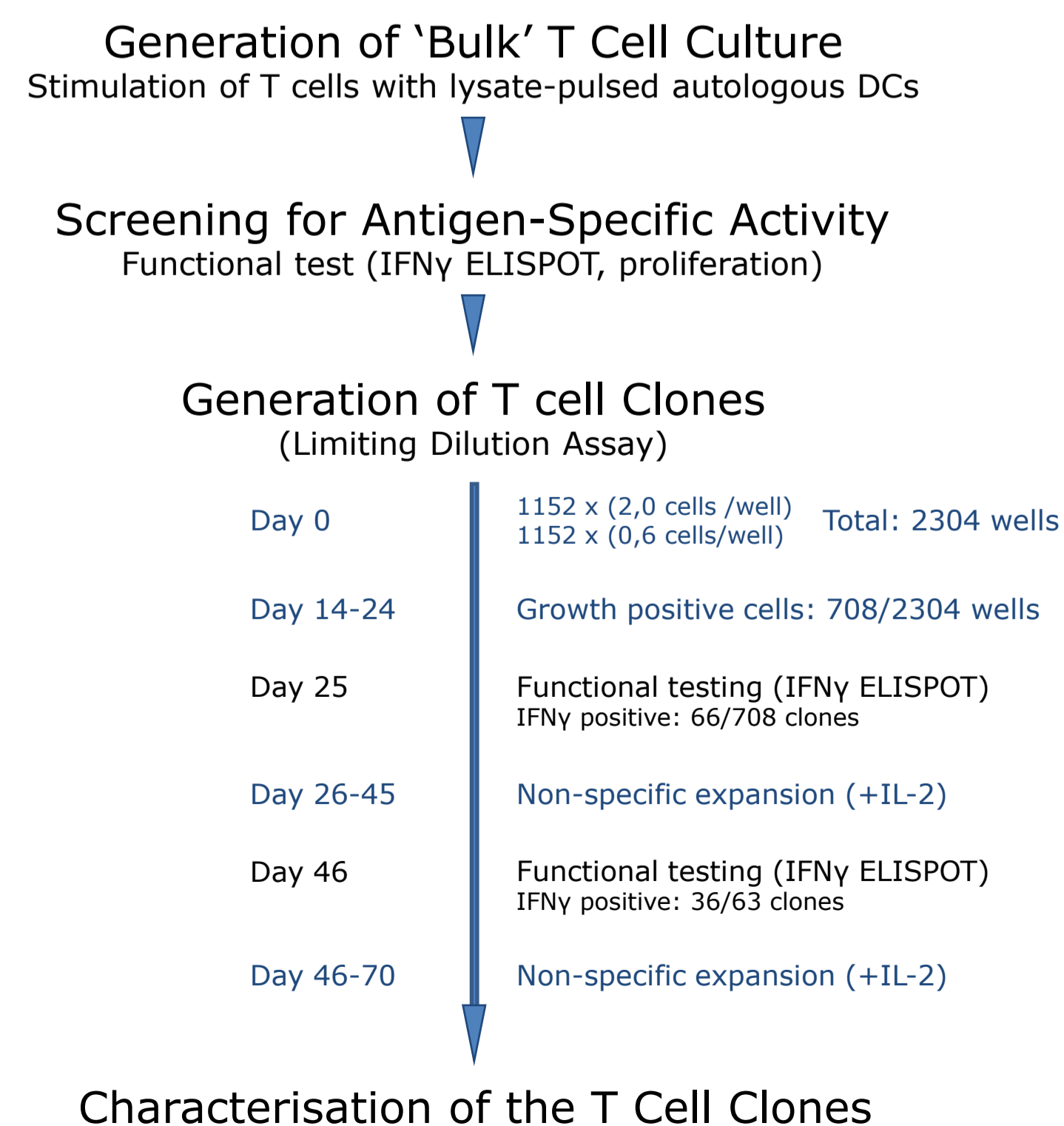
## Introduction

DanDrit's MelCancerVac® is a cancer vaccine that consists of patient's DCs loaded with cell lysate of a well-characterised allogeneic melanoma cell line (DDM1.7) expressing high levels of MAGE antigens. For such a vaccine to work, it is vital to ensure not only that the DCs are functionally immunogenic, but also that they present lysate-derived tumour antigens, a prerequisite for induction of specific anti-tumour T cell responses in the patient. Whilst methods for assessing the immunogenicity of DCs have been well-characterised and standardised, the demonstration of the potency by which lysate-derived tumour antigens are presented in MelCancerVac® has been difficult to measure due to the polytopic nature of the tumour lysate. In this study we describe our attempt to establish a potency assay for the vaccine.

## Experimental Approach

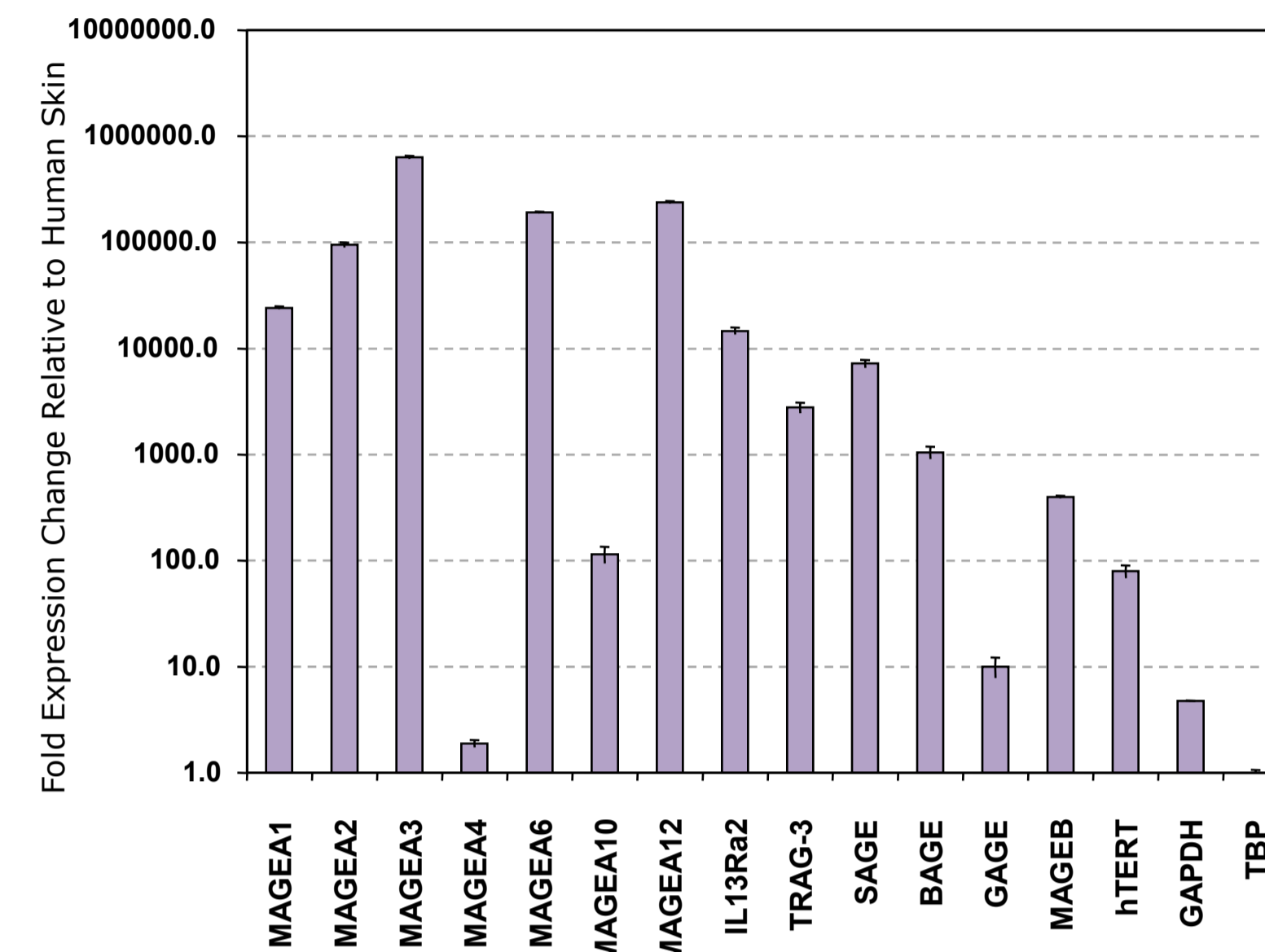
In order to establish a system to monitor tumour antigen presentation by MelCancerVac®, we generated T cell clones that react specifically to lysate-pulsed DCs. This was achieved by *in vitro* expansion of PBMC-derived T cells that are reactive to antigens presented by lysate-loaded autologous DCs. We used PBMCs from one of our lung cancer patients, who exhibited a measurable spontaneous immune response *in vitro* against MelCancerVac® (see figure 3A).

After total of three rounds of stimulation with MelCancerVac®, the specificity of the expanded T cell populations were tested (figure 3B & C), and T cells that specifically recognised antigens presented by MelCancerVac® were cloned by limiting dilution assay. The resulting clones were then expanded non-specifically by the addition of IL-2 and IL-4 for up to 70 days, during which time the phenotype and functionality of clones were analysed (summarised in figure 1).



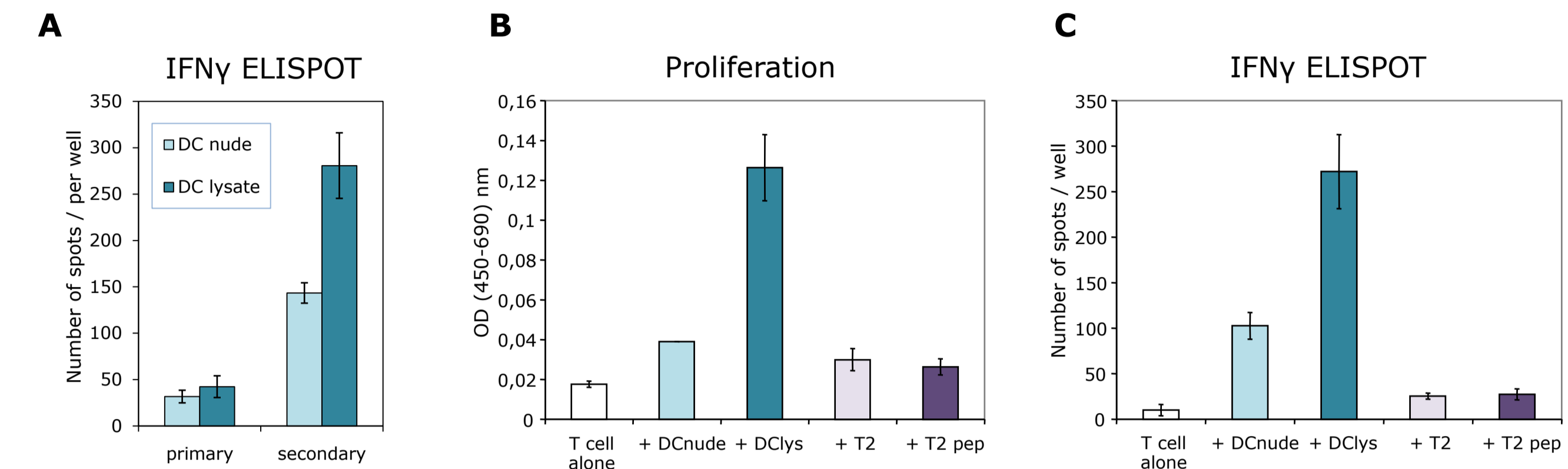
**Figure 1. Generation of MelCancerVac® - specific T cell clones.**

## Results



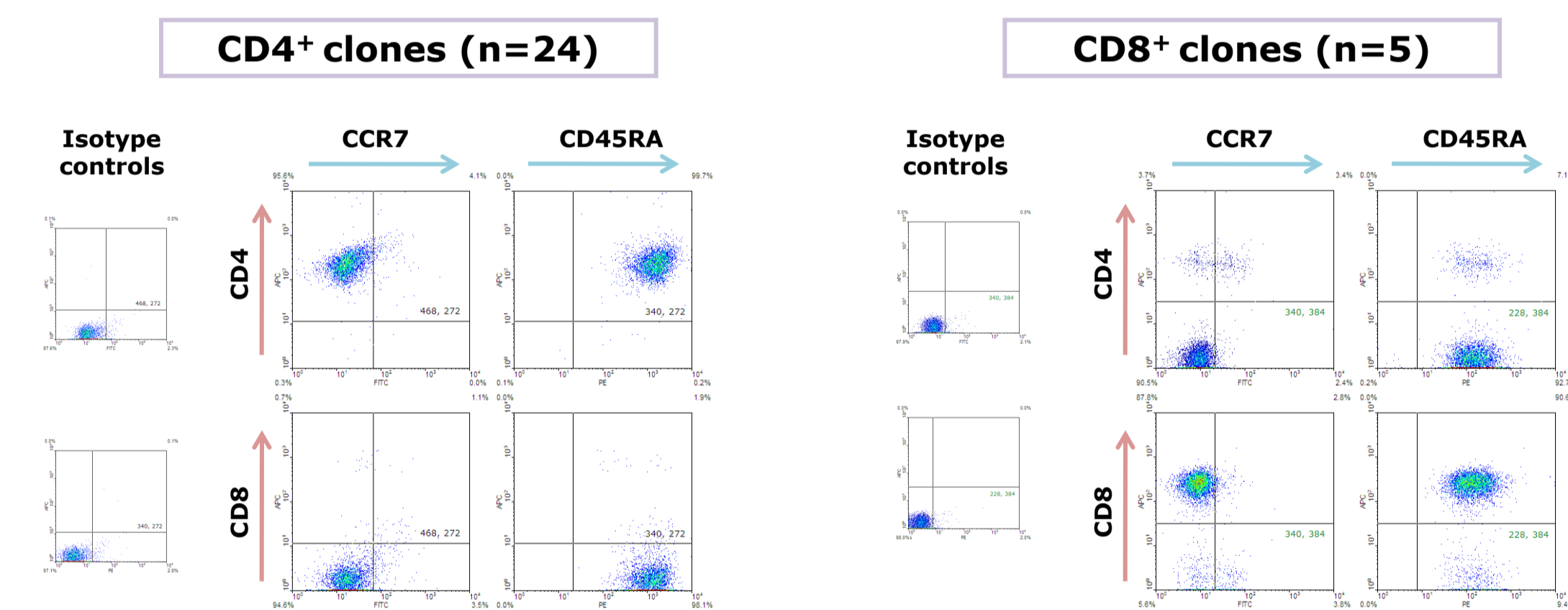
**Figure 2. Expression of tumour antigens in DanDrit's melanoma cell line (DDM1.7).**

Fold expression change of indicated tumour antigens in DDM1.7 cells relative to normal human skin total RNA is shown. The expression profile was generated by determining the expression level of each tumor antigen gene relative to a control gene (TBP, TATA box binding protein).



**Figure 3. T cell response against MelCancerVac® (before cloning).**

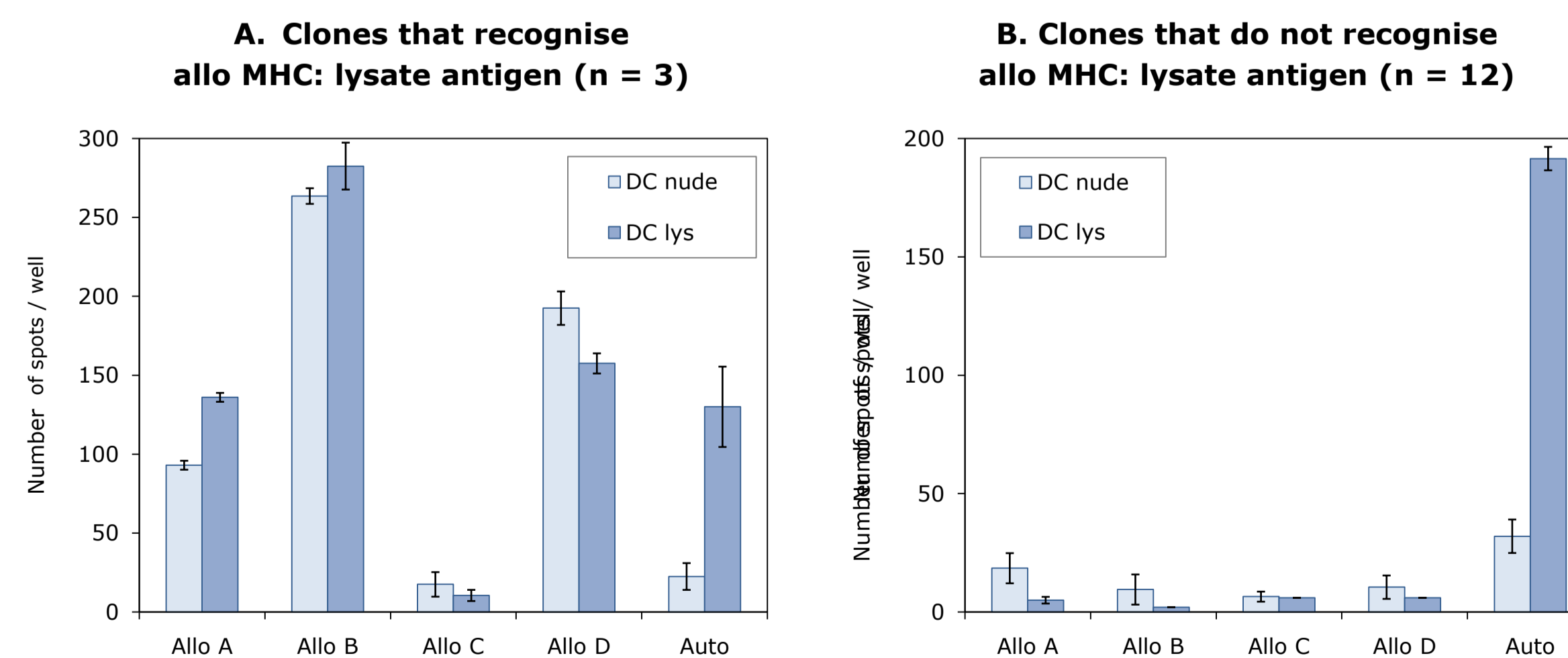
T cells isolated from PBMCs were co-cultured with DC +/- lysate (A, "primary"), or after 1 week of stimulation with lysate-loaded DCs (A, "secondary"), and the response was measured by IFN $\gamma$  ELISPOT. T cell response against lysate-loaded DCs or T2 cells loaded with known MAGE peptides was examined by measuring proliferation (B) or IFN $\gamma$  production (C) after two rounds of *in vitro* stimulation with lysate-loaded DCs.



**Figure 4. Majority of the clones are MHC class II restricted.**

T cell clones, expanded after the demonstration of IFN $\gamma$  response against MelCancerVac® (day 46 after limiting dilution, see figure 1), were examined for their surface phenotype by flow cytometry (day 62 after limiting dilution). Our of 29 clones tested, 24 of them were of CD4<sup>+</sup> subset, and the remaining 5 expressed CD8. One representative of each of these clones are shown.

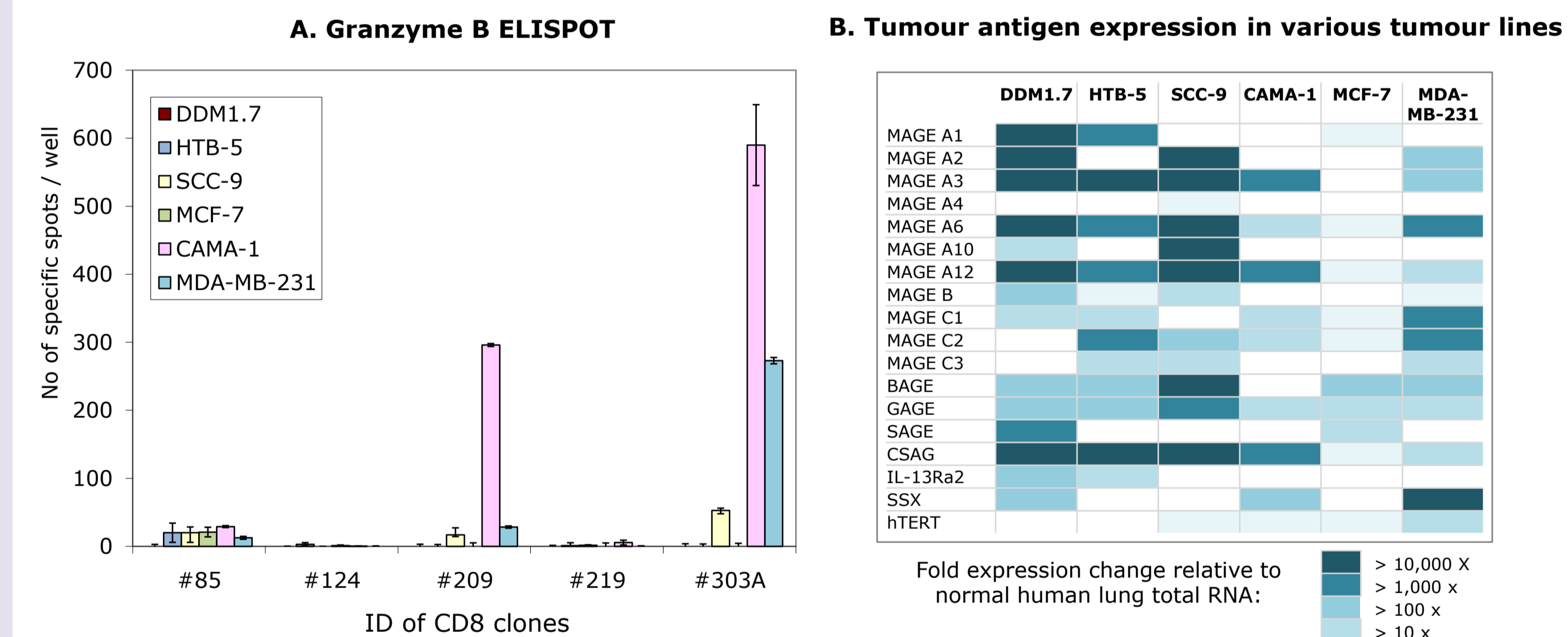
## Analysis of CD4<sup>+</sup> clones



**Figure 5. Recognition of lysate antigen presented by allogeneic DCs.**

CD4<sup>+</sup> clones were plated with DCs (+/- lysate) generated from autologous PBMCs ("Auto") or four different allogeneic donors ("Allo A-D"). T cell response was measured by enumerating IFN $\gamma$  secreting T cells by ELISPOT. One representative clone, each of A. clones that exhibited a partial response (in this case to allogeneic donor A) and B. clones that did not show any response to lysate antigen presented by allogeneic DCs, are shown.

## Analysis of CD8<sup>+</sup> clones



**Figure 6. CD8 clones recognise MAGE expressing tumour cell lines.**

A. CD8<sup>+</sup> clones were co-cultured with indicated cell lines at 10:1 ratio in an anti-granzyme B antibody-coated ELISPOT plate. After 4 hours of co-culture the plate was washed, and the frequency of granzyme-B secreting T cells were determined by standard ELISPOT assay. B. RT-PCR analysis of tumour antigen expression in the target cell lines. Shown are relative expression to normal human lung total RNA.

## Discussion & Conclusion

Our attempt to generate MelCancerVac®-specific T cell clones have resulted in generation of primarily CD4<sup>+</sup> subset. This may imply that the majority of T cell responses mounted against lysate-loaded DCs are directed towards MHC class II-restricted antigens. The remaining clones of CD8 subsets recognise various cell lines that express MAGE antigens. It remains unclear why these clones do not recognise the parental tumour line, DDM1.7 which has high expression of MAGE antigens. This, together with the observation that none of the known MAGE peptides were recognised by these T cells, it is possible that our clones are specific to antigens unrelated to MAGE.

Whilst our preliminary data are encouraging, further studies, including testing these clones against HLA-type matched donors, are required to examine whether these CD4 and CD8 clones generated can be used for the future potency assay.