ASSC Australian Skin and Skin Cancer Research Centre

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Pre-clinical development of antigen specific immunotherapy and strategies to overcome regulation in cutaneous malignant melanoma

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The University Of Queensland AUSTRALIA | DIAMANTINA INSTITUTE | TRI partnering for better health

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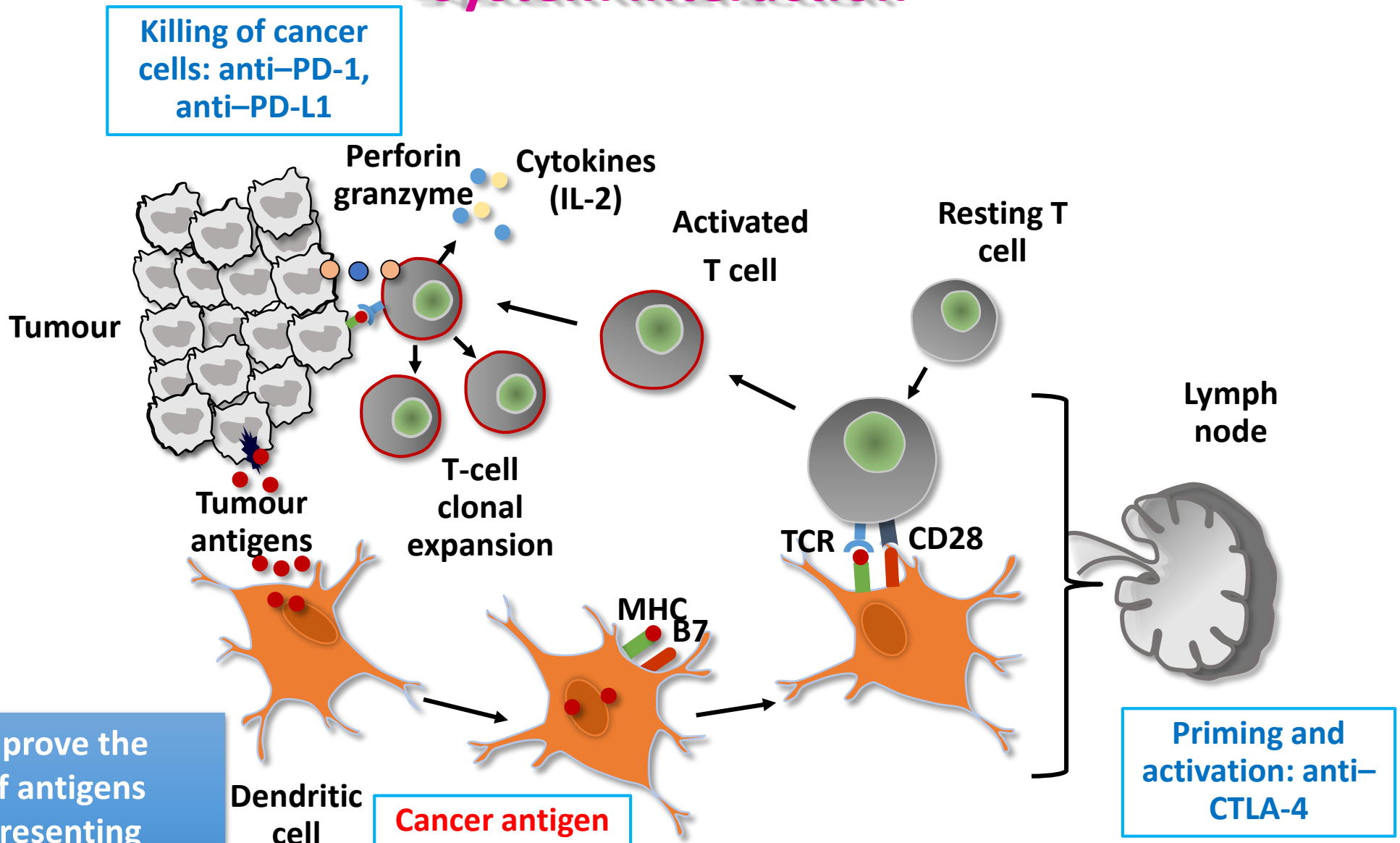
Background

- Despite the success of immune checkpoint inhibitors, a large number of patients with advanced cutaneous melanoma do not benefit from therapy.

- Early research indicates that a therapeutic combination of cancer vaccines with checkpoint inhibitors may lead to synergistic effects and higher response rates than monotherapy.

- Nevertheless, cancer vaccines of high potency and antigen-specificity are not available yet due to the limited efficacy of current strategies to target antigen and adjuvants to cross-presenting dendritic cells.

A Roadmap of Immunotherapy Agents in the Cancer:Immune System Interaction



Can we improve the delivery of antigens to cross-presenting dendritic cells?

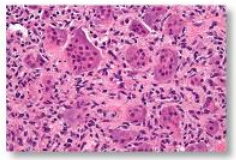
Aims

- To assess the efficacy of tumour antigen-loaded nanoemulsions targeting cross-presenting dendritic cells in combination with immune checkpoint inhibitors in a mouse model of melanoma.

- To assess the effects of this combination therapy on tumour microenvironment with particular focus on the immune regulatory network.

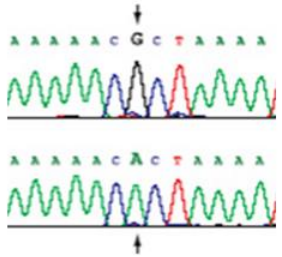
Exploitation of "T-cell druggable mutanome" with Clec9A-TNE

B16F10 mouse melanoma



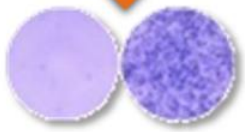
Expressed non-synonymous mutations

NGS

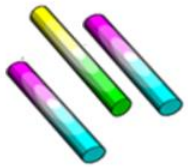


Validated immunogenic CD8 and CD8 neo-epitopes (Kreiter, Nature 2015)

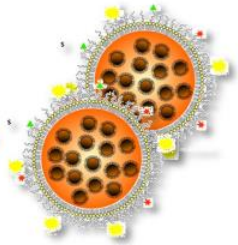
Gene	Mutated sequence used for vaccination
Gen1	IPHNPRVAVKTTNNLVMKNSVCLERDS
Polr2a	LAAQSLGEPATQITLNTFHYAGVSAKN
Tmtc2	QGVTVLAVSAVYDIFVFHRLKMKQILP
Zfr	AHIRGAKHQKVVTLHTKLGKPIPSTEP
Cep120	ELAWEIDRKVLHQNRLQRTPIKLQCFA
Malt1	FLKDRLLEDKKIAVLLDEVAEDMGKCH
Wdr11	NDEPDLDPVQELIYDLRSQCDAIRVTK
Kbtbd2	DAAALQMIIAYAYRGNLAVNDSTVEQL
Adamts9	KDYTAAGFSSFQKLRLDLTSMQIITTD
Pzp	AVKEEDSLHWQRPEDVQKVKALSFYQP
Gprc5a	FAICFSCLLAHALNLIKLVRGRKPLSW
Enho	MGAAISQGAIIAIVCNGLVGFLL
Dmrta2	EKYPRTPKCARCGNHGVVSALKGHKRY
Rragd	SHRSCSHQTSAPSPKALAHNGTPRNAI
Zzz3	KELLQFKKLKKQNLQQMQAESGFVQHV
Ilkap	RKGEREEMQDAHVSLNDITQECNPPSS
Cenpf	RVEKLQLESELNESRTECITATSQMTA



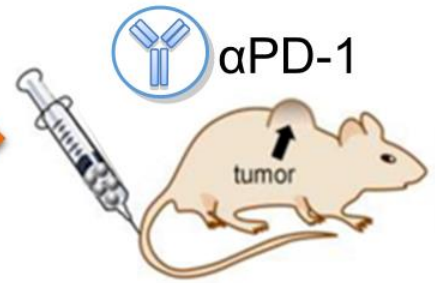
IFNγ ELISPOT



Selection of the most immunogenic neoepitopes In tumour-bearing mice



Nanoemulsions targeting Clec9A+ cross-presenting DCs



Ag-loaded Clec9A-TNE + anti-PD1 treatment of mice bearing B16F10 melanoma

Effects on immune regulatory tumour microenvironment

(Kelli MacDonald and Michelle Melino)

Regulatory T cells (Treg):

- Numbers
- Localization
- Activation status (PD-1, TIGIT, CD103, KLRG1, CD25, GITR, CD44, CD69, CD62L, CTLA4 and CD39).
- Function: cytokine production by intracellular cytokine staining, and *in vitro* suppression assays for antigen specific effector T-cell responses.

Myeloid-derived suppressor cells (MDSC):

- Two subsets: polymorphonuclear (PMN) and monocytic (M)-MDSC
  - PMN-MDSC - $CD11b^{+}Ly6G^{+}Ly6C^{lo}$
  - M-MDSC - $CD11b^{+}Ly6G^{-}Ly6C^{hi}$
- Enumeration
- Localization
- Function: expression of arginase, inducible NOS, TGF-β, IL-10 and COX2 IHC

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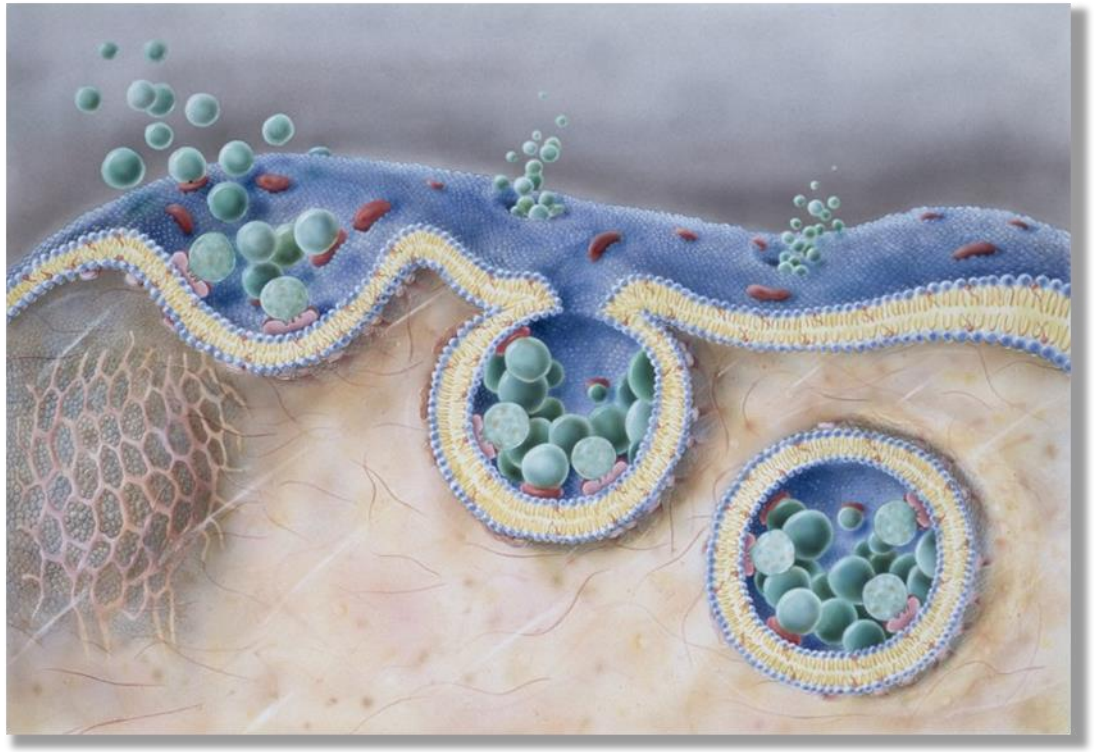
Effects of the treatment on blood-based exosomes: focus on immune regulatory proteins

Andreas Möller laboratory developed a rapid isolation and purification method for blood-based exosomes and will use this to determine exosome abundance, size and presence/absence of immune modulatory proteins on exosomes in the treatment and control mice.



Acknowledgements

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