Oral Presentations

Plenary symposium 3: B cells in immunooncology

03.05 TOWARDS A B CELL VACCINE FOR TUMOUR EXPRESSED SELF-ANTIGENS

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Background Monoclonal antibodies have huge potential in cancer therapy, but are expensive in production, storage, and delivery and have limited retention in the patient. To overcome this, active vaccination is a well-established procedure to induce long term production of antibodies. It also activates the response to multiple epitopes, which may confer additive effects on the target antigen. We try to use vaccination to induce antibodies targeting self-antigens expressed on tumours. We have shown that a conjugate vaccine can induce high titres of autoantibody specific to the tumour endothelial cell antigen Robo4, which is selectively expressed on tumour vascular endothelium, but not healthy vasculature. By covalently linking the extracellular domain of Robo4 to a common vaccine carrier protein, autoreactive B cells could recruit T cell help specific for the carrier. This protocol could efficiently induce specific anti-tumour vessel antibodies and suppress tumour growth in a Lewis Lung carcinoma (LLC1) mouse model. This project aims to optimise the vaccine-induced antibody response to Robo4 in LLC1 tumours and understand how the vaccine breaches immune tolerance in cancer.

Materials and Methods As most patients have pre-existing T cell memory to tetanus, we decided to use the non-toxic fragment C of tetanus toxin (FrC) as the carrier protein. Our data show that vaccination of mice with Robo4 genetically linked to FrC in adjuvant can efficiently induce the production of Robo4-specific antibodies.

Results The injection of Robo4-conjugate vaccine in adjuvant in a Lung Lewis carcinoma model did elicit Robo4-specific antibodies and retarded tumour growth. Further we discovered an increase in NK cell, $CD4^+$, and $CD8^+$ T cell and dendritic cell populations in tumours after Robo4 vaccination. LLC1 is a non-inflamed, poorly immunogenic tumour, resistant to immune-checkpoint therapy (ICT).

Conclusions Our data indicate that this vaccination strategy may promote immunogenic pathway activation and inhibit immunosuppressive factors present in LLC1 tumours, therefore having potential to improve the results of cancer therapy for ICT-resistant 'cold' tumours.

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03.06 UNDERSTANDING HOW EPITOPE BINDING INFLUENCES ANTIBODY DEPENDENT COMPLEMENT ENGAGEMENT FOR THERAPY OF B-CELL MALIGNANCIES

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Background Monoclonal antibodies (mAbs) have the capacity to combine targeted therapy with immune activation through recruitment of immune cells and complement proteins via their Fc-terminus. For treatment of B-cell malignancies, mAbs targeting the B-cell surface marker CD20 are well established; with Rituximab, Ofatumumab and Obinutuzumab being most commonly used in the clinic. Even though these three mAbs are all of the IgG1 subclass they differ in their effector functions, especially in their ability to engage the complement system for B-cell depletion. Efforts have been made to relate the functional properties of these mAbs to molecular characteristics to allow for informed decisions when designing next generation therapeutics.

Materials and Methods Real-time interaction analysis was performed with LigandTracer to deduct the binding mechanism of CD20 antibodies and complement protein on live cell lines and primary B-cells. Efficiency of complement dependent cell killing was evaluated *in vitro*.

Results Ofatumumab displayed the most stable binding to CD20 driven by bivalent target engagement which was independent of antibody concentration. Rituximab could overall engage more in bivalent binding compared to Obinutuzumab, but for both antibodies, stabilization of the interaction due to bivalency decreased with increasing antibody concentration, resulting in dose-dependent apparent affinities. The interaction strength of the first complement component C1q to the Fc-terminus of cell-bound mAbs correlated positively with degree of bivalent binding for the mAbs to CD20. Kinetic analysis revealed two multivalent interaction components that represent C1q binding to low- and high-order IgG Fc-oligomers on the cell surface. The latter interaction component was characterized by more stable C1q capture and most dominant for Ofatumumab. Target cell killing through antibody dependent complement activation was more efficient with strong bivalent target engagement and high C1q binding stability.

Conclusions Taken together this implies that bivalent target engagement helps cluster CD20 on the cell surface leading to beneficial arrangement of IgG Fc for stable C1q capture through optimization of avidity effects, which translates to efficient activation of the complement cascade. These findings are strongly supported by recent structural data in the field and add to the understanding on how the binding mechanism influences immune activation for CD20 mAbs.

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