Novel highly efficient cell based immunization platform for antibody generation against intractable drug targets

Debby Kruijsen, Jeanette Leusen#, Nienke Smits and Jos M.H. Raats

ModiQuest Research B.V., Oss, The Netherlands, #Laboratory of immunotherapy, UMC Utrecht, The Netherlands

Introduction

ModiQuest Research presents a novel platform to generate high affinity antibodies specific for intractable drug targets such as low immunogenic membrane-spanning proteins.

CD20 is a low immunogenic type III transmembrane protein of which the extracellular domain consists of two small loops. Rituximab, a CD20-specific monoclonal antibody, has been widely used in the clinic for the depletion of B cells to treat various forms of cancer and autoimmune diseases. Since not every patient responds to rituximab, the availability of anti-CD20 monoclonal antibodies with improved efficacy (e.g. ADCC, CDC) is desirable. In order to generate anti-CD20 antibodies with a broad epitope specificity and improved efficacy, the ModiVacc™ cell immunization approach was applied.

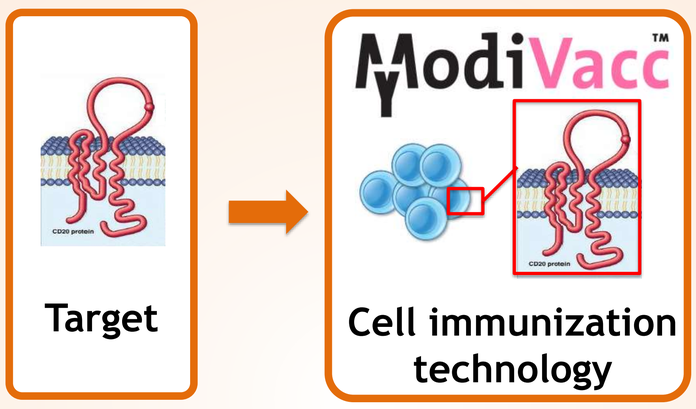
Objective

Generation of monoclonal antibodies specific for the native conformation of CD20 with high affinity, CDC and ADCC activity using the ModiVacc™ approach.

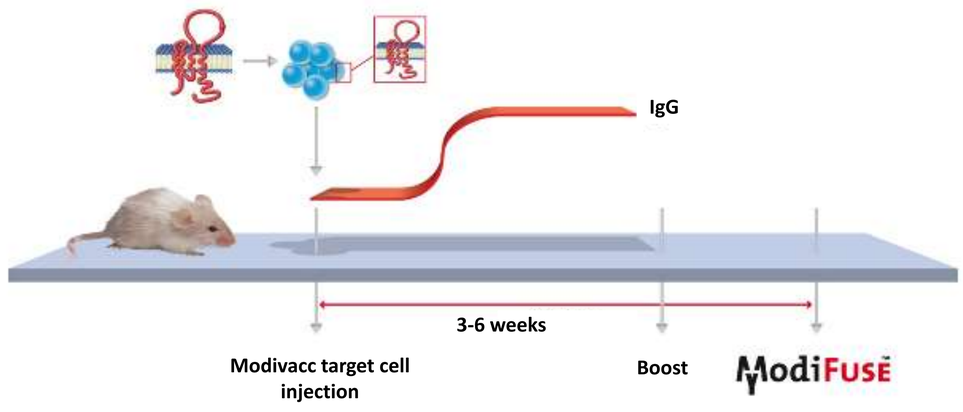
Methods

Cell line generation and immunization

In order to generate monoclonal antibodies against CD20, a proprietary mouse tumor cell line, ModiVacc™ (1), stably expressing CD20 was generated. ModiVacc™-CD20 cells were cultured, selected by FACS for target expression and subsequently used to immunize mice.



Spleen cells of ModiVacc™-CD20-immunized mice with a target-specific titer were used to generate hybridomas. Resulting hybridomas were analyzed for reactivity towards ModiVacc™-CD20 cells and negative control cells using FACS. Target-reactive hybridomas were subjected to a sub-cloning procedure and subsequently screened for target reactivity in ELISA, FACS, CDC, ADCC and affinity.



Results

Live cell immunization

To generate antibodies specific to the extracellular domain of CD20, mice were immunized with ModiVacc™-CD20 cells at day 0. The immunization of live ModiVacc cells did result in an initial expansion of the tumor cells in the mice and induced a very strong immune response against the membrane protein-expressing ModiVacc-cells. This immune response ultimately resulted in the clearance of the expanding tumor cells after 10-14 days (Figure 1).

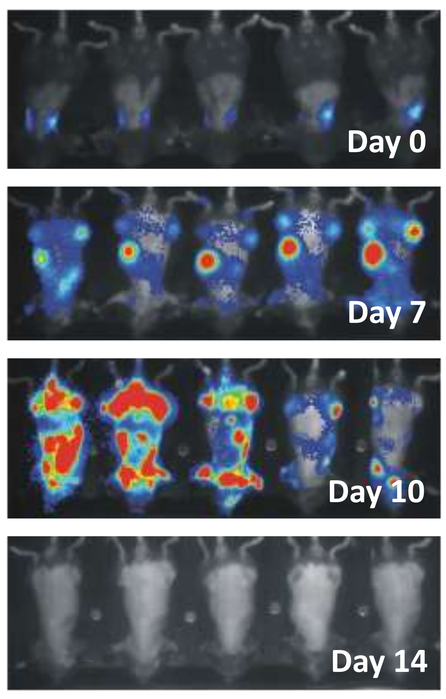


Figure 1. Bioluminescence image of immunized mice. After initial outgrowth, ModiVacc tumor cells are cleared from the mice.

Results

Mouse anti-CD20 antibodies

To day 14 after immunization with with ModiVacc™-CD20 cells blood was withdrawn for serum titer analysis using FACS. A high CD20-specific antibody titer was observed at day 14, when compared to day 0 (Figure 2).

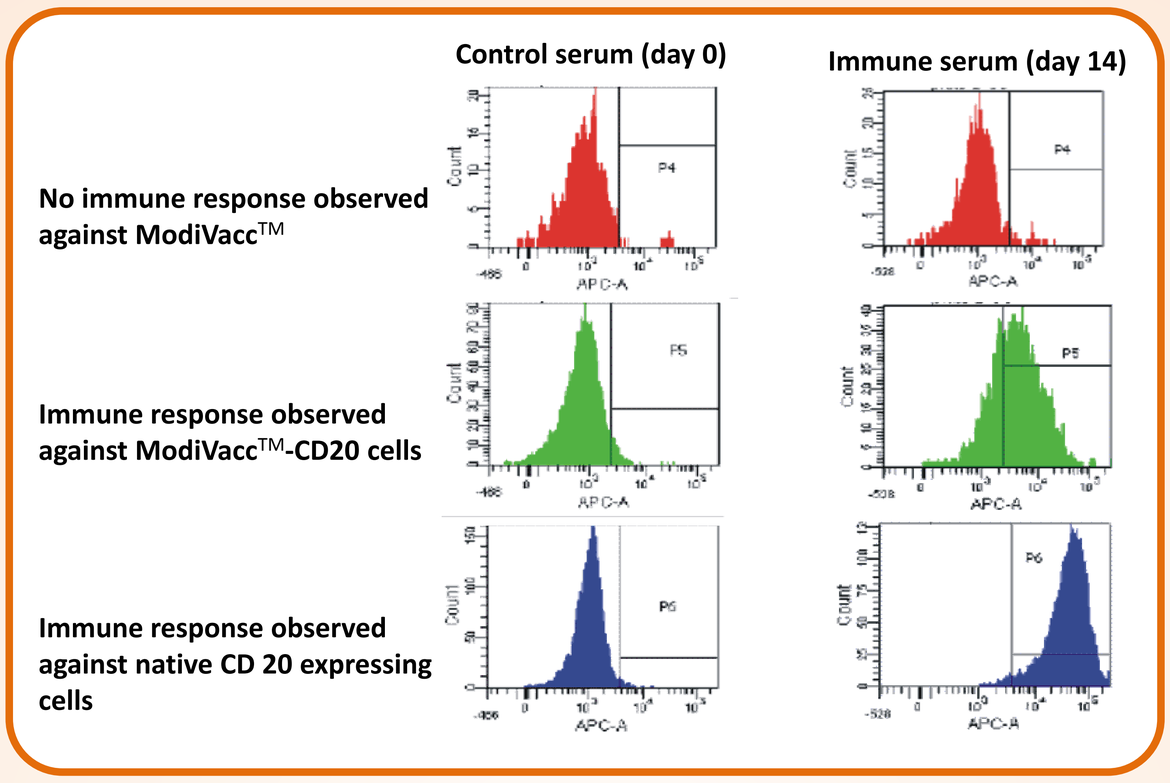


Figure 2. High CD20-specific titer was observed at day 14 after immunization. Blood was withdrawn and IgG Ab titers were determined with a FACS-based screening assay. ModiVacc™ cells, CFSE-labeled ModiVacc™-CD20 cells and LawaCell-labeled Daudi cells expressing CD20 were mixed at 1:1:1 ratio and incubated with sera from mice. Binding of CD20 was revealed with APC-labeled anti-mouse IgG Abs.

To analyze the diversity and functionality of the induced antibodies, monoclonal antibodies were generated from spleen cells of the best responder mouse. The obtained hybridomas were screened for target reactivity in ELISA and FACS. Next, CD20-reactive hybridomas were further characterized for sequence diversity, ADCC and CDC. This resulted in 17 monoclonal antibodies with a major sequence diversity in the CDR3 region, with comparable ADCC capacity and varying CDC capacity of which 8 were selected with distinct binding characteristics for further clinical development (Table 1).

Name	mAb 1	mAb 3	mAb 4	mAb 5	mAb 6	mAb 7	mAb 10	mAb 17
CDC	+++	+	+	+	++	++	+++	++
ADCC	+++	++	++	++	+++	+++	+++	++
Isotype	IgG2a	IgG2b	IgG2b	IgG2b	IgG2b	IgG2b	IgG2a	IgG3

Table 1. Monoclonal antibodies with distinct binding characteristics selected for further clinical development.

The antibodies displayed in Table 1 were subjected to affinity analysis. Antibody affinities were in the range of $3.8x10^{-9}$ M to $8.9x10^{-10}$ M. The relative affinity curves of 4 antibodies are displayed in Figure 2.

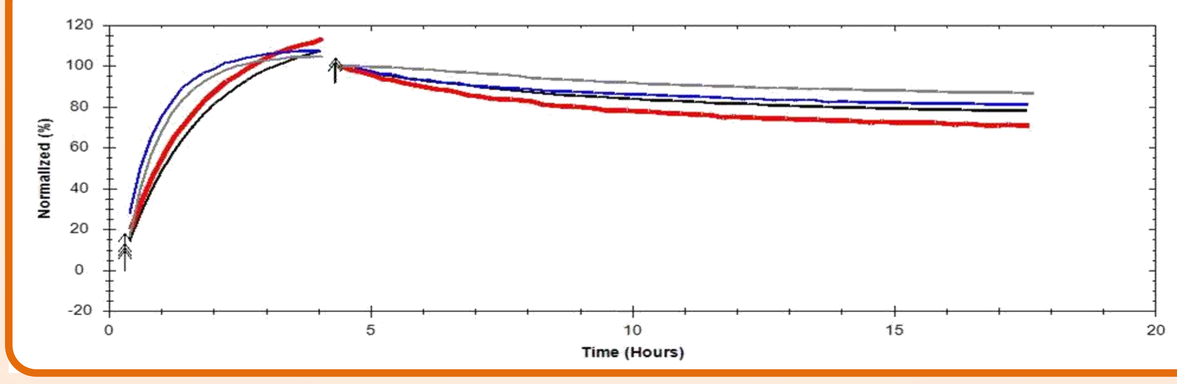


Figure 3. New CD20 mAbs with affinities in the nanomolar range. Real-time binding kinetics using the Ligand Tracer technology. Binding of FITC-labeled CD20 mAbs was performed on SKBR3-CD20 cells. mAbs were incubated at 10 nM for 3 hours, followed by dissociation measurement for several hours with medium.

Conclusions

The novel ModiVacc™ approach is proven to be a highly successful approach in generating antibodies to difficult low immunogenic membrane proteins. Next to the above described CD20 antibodies, large panels of high affinity antibodies recognizing a broad variety of epitopes have already been generated to a number of difficult antigens (e.g. CD89, CD38, Her2, human and mouse LAIR, PDGFR, PDL1, DCIR, YδTCR). The ModiVacc™ method has many advantages compared to DNA or virus-like particle immunization approaches.

Acknowledgements

[1] The ModiVacc cell line is in-licensed from UMC Utrecht, The Netherlands