The standard therapy used for the treatment of metastatic prostatic cancer, androgen ablation, fails to induce programmed cell death in androgen- independent prostatic cancer cells. However, in vitro, androgen-independent cancer cells can be triggered (by calcium ionophores) to undergo apoptosis, i.e., the pathway needed for this programmed cell death is retained. Recent evidence indicates that a highly metastatic, androgen-independent AT-3 tumour cell line expresses a tumour-specific antigen (PSA) which is the target of an idiotype network. We are currently performing cross-blocking RIA to determine whether these Ab2 recognize different epitopes in anti-PSA MAb binding pocket and not for the CH2 (outside pocket) of IgG1.

For the generation of anti-idiotype antibodies (Ab3) resembling Ab1 (Ab1'), New Zealand rabbits were immunized with Ab1-KLH. Serum samples collected after 4 months were used to test anti-idiotype antibodies. Anti-idiotype antibodies were isolated from the serum by affinity chromatography and used as immunogen. BALB/c mice were immunized with Ab1-KLH, which specifically reacts with PSA and their splenocytes were fused with SP2/0 myeloma cells. In 3 fusions, 6 anti-idiotype antibodies specifically reacting with anti-PSA MAbs were isolated from more than 300 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAb binding pocket and not for the C2 (outside pocket) of IgG1. Furthermore, these Ab2 were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAbs IgG. All Ab2 showed clear binding to anti-PSA MAb in nodal, tissue culture and tumor cell lines, whereas binding was observed only to tumor cell lines and not to normal cell lines, indicating that Ab2 recognize a conformational epitope compromised of heavy and light chain, and not a structural epitope outside anti-PSA MAb binding site. I.e. they have also initiated the functional characterization of these Ab2, i.e., whether they are able to induce antibodies capable to compete with the parental anti-PSA MAb. For the generation of anti-anti-idiotype antibodies (Ab3) resembling Ab1 (Ab1'), New Zealand rabbits were immunized with Ab1-KLH. Serum samples collected after 3 months were used to test anti-anti-idiotype antibodies. Anti-anti-idiotype antibodies were isolated from the serum by affinity chromatography and used as immunogen. BALB/c mice were immunized with Ab1-KLH, which specifically reacts with PSA and their splenocytes were fused with SP2/0 myeloma cells. In 3 fusions, 6 anti-anti-idiotype antibodies specifically reacting with anti-PSA MAbs were isolated from more than 300 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAb binding pocket and not for the C2 (outside pocket) of IgG1. Furthermore, these Ab2 were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAbs IgG. All Ab2 showed clear binding to anti-PSA MAb in nodal, tissue culture and tumor cell lines, whereas binding was observed only to tumor cell lines and not to normal cell lines, indicating that Ab2 recognize a conformational epitope compromised of heavy and light chain, and not a structural epitope outside anti-PSA MAb binding site. I.e. they have also initiated the functional characterization of these Ab2, i.e., whether they are able to induce antibodies capable to compete with the parental anti-PSA MAb. For the generation of anti-anti-idiotype antibodies (Ab3) resembling Ab1 (Ab1'), New Zealand rabbits were immunized with Ab1-KLH.