

Marlon Romano Veldwijk  
Dr. sc. hum.

## **New Strategies for the Therapy of Sarcomas Using Recombinant Adeno-associated Virus 2 Vectors that Contain a Suicide Gene**

Geboren am 13.11.1973 in Amsterdam (NL)  
Reifeprüfung im Juni 1992 in Lelystad (NL)  
Vordiplom im August 1993 an der Freien Universität (VU) von Amsterdam (NL)  
Diplom im August 1997 an der Freien Universität (VU) von Amsterdam (NL)

Promotionsfach: Innere Medizin  
Doktorvater: Priv.-Doz. Dr. med. S. Fruehauf

Adeno-associated virus 2 (AAV-2) vectors are used for clinical gene therapy of hereditary diseases and at this stage for preclinical cancer gene therapy. In this work a high susceptibility of some solid tumors to an rAAV-2 vector containing the *humanized green fluorescent protein (hGFP)* gene was shown. Among a series of primary cells and eight solid tumor cell lines infected with these AAV-2 supernatants, the highest infection rates (functional titer: 200 IU/cell) were observed in soft tissue sarcoma cells (HS1; mean: 96% hGFP<sup>+</sup> cells) and in breast cancer cells (T47D; mean: 83%, MCF-7; mean: 85%); other cell lines (1 ovarian tumor, 1 germ cell tumor, 1 osteosarcoma and 2 small cell lung cancer) were less permissive (3% - 12%); CD34<sup>+</sup> peripheral blood progenitor cells showed the lowest transduction rates (max. 4%). These data suggest that the sarcoma and breast cancer cells are the most suitable candidates for further development of AAV-2 tumor suicide gene therapy. The first requirement for using these vectors in cell kill experiments was the construction of new rAAV-2 vectors containing a suitable suicide gene. The *thymidine kinase (TK)* gene was chosen in combination with the prodrug ganciclovir. Three novel TK-containing vectors were cloned. Since some of the new vectors contained no marker gene (*GFP*) and therefore did not allow fluorescence-based titration, a highly optimized titration method was established based on the real-time quantitative polymerase chain reaction so that rAAV-TK vectors which did not contain a fluorescent marker gene could be titrated accurately. Four sarcoma cell lines were chosen (HS-1, HT1080, RDES and SK-N-MC) and a complete eradication of all rAAV-TK/eGFP transduced tumor cells was shown following exposure to ganciclovir (2.5 µg/ml) *in vitro* while at this dose level >95% of mock-transduced tumor cells survived. Xenotransplantation tumor models for these human sarcoma cell lines were established. In proof of principle experiments mice transplanted with rAAV-TK/eGFP-transduced and ganciclovir-exposed tumor cells survived >5 months while in the non-transduced group all mice had died 1 month after inoculation. These data hold promise for a future clinical application of AAV-2-based suicide gene therapy.