Rene Vogels stichting
Mirjam van de Velde

Individual treatment for the prevention of vincristine induced peripheral neuropathy in children with cancer

Background

Vincristine (VCR) is a commonly used chemotherapeutic agent for the treatment of pediatric cancer. The most important side effect is VCR induced peripheral neuropathy (VIPN). This is a dose limiting toxicity, leading to a peripheral and mainly sensory-motor neuropathy. Clinically a child with VIPN may suffer from paresthesia and neuropathic pain, muscle weakness and/or constipation. Currently there are no sufficient treatment options available for VIPN. There are multiple factors that play a role in the development of VIPN. There is a dose-dependent relation between VIPN and VCR. Furthermore, it was shown that concurrent use of azoles and VCR lead to an increase and VIPN. One of the patient-derived factors that is associated with VIPN are pharmacokinetics (PK) of VCR. However, there are large inter-individual differences. Moreover, there is a clear racial difference, in which Caucasian children are more often and more severely affected then non-Caucasian children. This is most likely due to genetic differences between these groups. Another genetic factor associated with differences in susceptibility to VIPN is a single nucleotide polymorphism (SNP) in the promoter region of the CEP72 gene. The current project is part of a large multicenter trial studying the relation between administration method of VCR and VIPN.

Method

During the current research project we aim to study the relation between occurrence and severity of VIPN on the one hand and PK and genetic factors on the other hand. Therefore, we have analyzed samples that were collected during the multi-center trial studying the effect of administration method of VCR in St. Jude Children's Research Hospital (St. Jude) in Memphis, Tennessee. For the analysis of pharmacokinetic factors we used Stochastic Approximation Expectation-Maximization (SAEM) in a two-compartment model with Monolix. For the analysis of DNA samples we used whole-exome sequencing. Furthermore, during this project we studied the influence of the CEP72 gene on the sensitivity for VCR. We used specific B-cel childhood leukemia cellines (697 and Nalm 6), on which CRISPR/Cas was used to create a knock-out of CEP72. Following this we used Sanger sequencing and Western blotting to see if we were able to create this knockout. The sensitivity of the cellines on VCR was tested by dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

Results

For the analysis of PK samples we have used data of 35 children with in total 473 samples. The preliminary results are shown in Table 1.

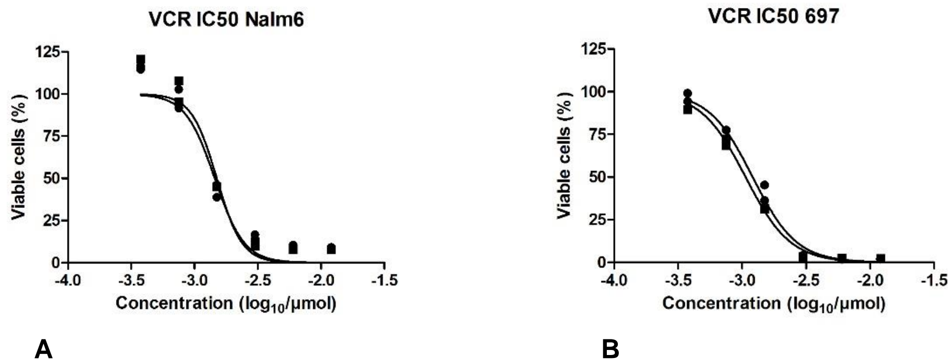
Table 1: Summary of pharmacokinetic results of vincristine

	Push administration			1-hour administration			Total		
	Min	Median	Max	Min	Median	Max	Min	Median	Max
Cl (L/hour/m^2)	12.19	28.2	41	16.4	24.3	48	1.77	25.7	70.4
V1 (L/m^2)	3.9	39.5	337	9.98	23.1	97.8	6.53	31.6	186
Q (L/hour/m^2)	14	14	904	18.4	32.7	77.5	17.8	86.1	864
V2 (L/m^2)	144	144	2210	43.4	121	694	78.9	390	1330

Cl: clearance, V1: volume of the central compartment, Q: intercompartmental clearance, V2: volume of the peripheral compartment, min: minimum, max: maximum

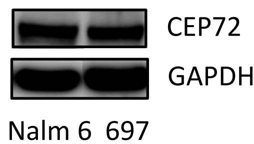
These results are comparable with previous results. Currently we are working on the analysis between clearance, volume of the central compartment (V1), intercompartmental clearance (Q) and volume of the peripheral compartment and the development and severity of VIPN, of which results will follow shortly. For the study question regarding the association between genetic factors and the development of VIPN, DNA samples of 85 children are analyzed, of which the statistical analysis are currently executed. We expect these results within the upcoming months.
The influence of CEP72 on the sensitivity of VCR in the 697 and Nalm6 cellines before the CRISPR/Cas knockout are shown in Figure 1 and 2. These Figures shows the sensitivity of these cellines in the presence of CEP72. Furthermore, the Westernblot in Figure 2 confirms the expression of CEP72 in these cellines.

Figure 1: Results of the MTT assay prior to CRISPR/Cas knock-out



VCR: vincristine, IC50: half maximum inhibiting concentration, squares and rounds represent individual measurement on two different days and timepoints.

Figure 2: Results of Westernblot prior to CRISPR/Cas knock-out



By using nucleofection a CRISPR/Cas knock-out was created. By Sanger sequencing it was confirmed that two clones were developed in the 697 celline, which are heterozygous for the CEP72 knock-out. We expect a 50% reduction in the activity of this protein within these cells. Currently these cells are growing until there is a sufficient number to test by using MTT and Westernblot the effect of this knock-out on the sensitivity of VCR. These analysis will be done in the upcoming months.

My personal results of the current project were the expansion of my research abilities. I have learned how to build a PK model based on SAEM in Monolix. Furthermore I have learned a number of laboratory skills, such as the execution of an MTT assay and Westernblots and new techniques such as the creation of knock-out by use of nucleofection and CRISPR/Cas. Currently I learn a lot by doing the analysis of the WES results in relation to VIPN. Overall, my exchange with St. Jude was a very inspiring and helpful experience. It is something I can take with me throughout my entire career.