Unit Pharmacology of Cancer Treatment (D0200 / E120)

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The unit consists of the working groups of W. Jens Zeller and Rüdiger Port.

Principal research topics of W.J. Zeller are sensitizing and protective factors in cancer chemotherapy.

Close cooperation exists with Priv.-Doz. Dr. S. Fruehauf and Prof. Dr. A.D. Ho of the Department of Internal Medicine V of the University of Heidelberg; the aim of this cooperation is an optimization of high-dose chemotherapy with blood stem cell support using preclinical models (stem cell expansion, cytostatic drug resistance gene transfer) and experimental therapy of chronic myelogenous leukemia.

R. Port’s work focuses on pharmacokinetic and pharmacodynamic modelling.

Protection of hematopoietic cells from chemotherapy-induced toxicity by multidrug resistance-1 gene transfer followed by analysis of retroviral insertion sites into the human genome


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The human multidrug resistance-1 (MDR1) gene is a potential selectable marker gene for hematopoietic stem cell gene therapy of malignant or non-malignant conditions. Thus far no clear in vivo myeloprotection has been shown following retroviral MDR1 gene transfer to human mobilized peripheral blood progenitor cells (PBPC) and pilot clinical trials with murine retroviral vector have failed to demonstrate the feasibility of this gene therapy approach due to low transduction efficiencies of human hematopoietic stem cells and insufficient expression of the MDR1 gene in vivo. Therefore, more extensive preclinical characterization and the use of more adequate preclinical models, such as the non-obese diabetic/severe combined immunodeficient (NOD/SCID)-human assay for stem cell gene therapy are necessary. We used a retroviral vector SF91m3 containing the MDR1 cDNA, which was improved for transcriptional strength and RNA processing in hematopoietic cells for transduction of human CD34 selected PBPC.

Clinical scale human PBPC cell grafts transduced with this vector were transplanted into NOD/SCID mice. We showed that retroviral transfer of the MDR1 gene to human mobilized PBPC with marrow repopulating ability can confer resistance to MDR1-dependent chemotherapy in vivo. Furthermore, an expansion of MDR1-transduced NOD/SCID-engrafting cells with high-level P-glycoprotein expression after a chemotherapy challenge was demonstrated. The median level of long-term human leukocyte chimerism in NOD/SCID mouse BM was 56%-78%. The level of human cell engraftment was similar in mice receiving MDR1-transduced cells and mock-transduced cells, suggesting that with the transduction protocol used and the improved SF91m3 vector no myeloproliferative syndrome (MPS) had occurred during the eight weeks observation period. The median gene transfer rate to NOD/SCID mice-repopulating human cells derived from PBPC (SRCs) ranged between 9.4%-12.3%. A median of 59% SF91m3-transduced cells clearly expressed the transgene and a tight correlation was observed between gene marking and expression.

A sublethal dose of paclitaxel was administered to chimeric mice. The proportion of human cells remained significantly higher in MDR1-transduced than in mock-transduced mice, suggesting that MDR1-transduced cells had been chemoprotected. In comparison to untreated MDR1-transduced mice a significant 1.4-1.8-fold increase in the proportion of gene marked or P-glycoprotein expressing human cells was noted in paclitaxel treated mice. In group 2 a similar human cell engraftment was found as reported for group 1. With the aim of dose intensification tighter intervals between transplantation of MDR1-transduced PBPC and the first chemotherapy course are under investigation.

Recent reports on retroviral vector insertion mutagenesis in mice and humans have created intense interest to characterize vector integrations on a genomic level. To investigate potential side effects which can be caused through these integrations we have identified retroviral integration site. We studied the above described retrovirally transduced human PBPC with bone marrow repopulating ability in immune-deficient mice. We used a ligation-mediated PCR followed by sequencing of vector integration sites, and found a multitude of simultaneously active human stem cell clones 8 weeks after transplantation. Interestingly, vector integrations occurred with significantly increased frequency into chromosomes 17 and 19 and into specific regions of chromosomes 6, 13 and 16 although...
the majority of chromosomes were targeted. Preferred genomic target sites have previously only been reported for wild-type retroviruses. Our findings reveal for the first time that retroviral vector integration into human marrow-repopulating cells can be nonrandom (P = 0.00037). Knowing which preferential integration sites are used in stem cells may help to understand mechanisms of integration and eventually allow vectors to be targeted to preferred sites. This may help to avoid insertional mutagenesis and reduce the genotoxicity of retroviral vector-mediated gene transfer which has recently been reported for mice and humans.

**Publications (\* = external co-author)**


**Suicide gene therapy of sarcomas and mesotheliomas using novel recombinant adeno-associated virus 2 vectors**

M.R. Veldwijk, S. Berlinghoff, S. Laufs, S. Fruehauf* and W.J. Zeller

*Department of Internal Medicine V, University of Heidelberg

Although great efforts have been made to improve conventional therapy for sarcoma and malignant mesothelioma, the median survival time of these entities after appearance of clinical symptoms stays poor. Effective (loco-regional) therapy using viral vectors that contain a suicide gene may be an alternative treatment strategy.

For sarcoma, we previously reported the highest susceptibility for recombinant adeno-associated virus 2 (rAAV-2) vectors in human connective tissue sarcoma cells (line HS-1). Now we confirm our findings in five further human sarcoma cell lines: fibrosarcoma (line HT-1080), Ewing’s sarcoma (line RD-ES), Askin’s tumor (line SK-N-MC), rhabdomyosarcoma (line A-204) and soft tissue sarcoma (line WSKL-1). Furthermore, we found that rAAV-2 also achieved both high transduction rates and GFP expression levels in human mesothelioma (lines: H-Messo-1, MSTO-211H and NCI-H-28). Among rAAV-2-constructs containing different promoters, after transduction, the vector with the elongation factor 1-alpha (EF1α) promoter showed the highest expression rates in all these cell lines. Several new thymidine kinase (TK) gene-variably containing vectors under control of either the cytomegalovirus or the elongation-factor 1 alpha (EF1α) promoter were cloned and tested. A higher expression level of the transgene was observed in the sarcoma and mesothelioma cell lines when using the EF1α-suicide gene-containing vectors. For the sarcoma cell lines we were able to show a complete eradication of all rAAV-EF1α-TK/eGFP (contains a thymidine kinase/enhanced green fluorescent protein fusion protein) transduced tumor cells following exposure to ganciclovir (2.5 μg/ml) in vitro, while at this dose level >90% of mock-transduced tumor cells survived. Using mesothelioma cell lines a nearly complete eradication (>2 log) of transduced and GCV-treated cells could be obtained using this vector.

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**Publications (\* = external co-author)**


**Potentiation of imatinib activity in chronic myelogenous leukemia cells by farnesyltransferase inhibitors.**

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*Internal Medicine V, University of Heidelberg, Heidelberg, Germany

Farnesyltransferase inhibitors (FTI) were reported to inhibit growth of chronic myelogenous leukemia (CML) cells. We investigated the activity of FTI L-744,832 and LB42918 alone and in combination with imatinib (formerly termed...
STI571), a tyrosine kinase inhibitor known for its efficacy in treatment of CML, in CML cell lines and primary CML cells. LB42918 was more potent with ED50=3.5±0.8 µM (mean±S.D., n=3) in the BCR-ABL+ cell line EM-3 vs. ED50=25.4±1.9 µM for L-744,832 after 48 hrs of treatment and assessment by MTT assay. The growth of BCR-ABL+ K562 and LAMA-84 cells was not measurably inhibited by L-744,832 doses up to 25 µM. Similarly, the growth of K562 was not affected by 25 µM of LB42918. However, for LB42918 in LAMA-84 cells an ED50 value of 29.7±10.5 µM could be determined. In the FTI-sensitive cell line EM-3 combined effects of both FTI with imatinib were assessed using the median-effect method of Chou and Talalay. Both combinations were strongly synergistic with combination index (CI) values of 0.47±0.18 and 0.67±0.15 at ED75 for imatinib+L-744,832 and imatinib+LB42918 respectively. Using the Annexin V/propidium iodide assay we observed a strong increase of the apoptotic cell fraction in EM-3 cells treated for 24 hrs by the combination imatinib+L-744,832 as compared to treatment with each drug alone. The influence of imatinib and L-744,832 on CFU-GM colony formation of primary CML cells obtained from 4 patients was investigated and a mutual enhancement of inhibitory effects was observed again. Interestingly, in the FTI-resistant cell lines K562 and LAMA84 a trend to lower ED50 values of imatinib was determined when up to 5 µM of L-744,832 or LB42918 were added, signifying potentiation of imatinib (see Table for imatinib+L-744,832, all doses are given in µM).

ED50 of imatinib alone +1 µM L-744,832 +5 µM L-744,832
K562 0.65±0.15 0.55±0.21 0.39±0.08
LAMA-84 0.42±0.16 0.23±0.12 0.18±0.09

The strong variation of FTI activity in different CML cells derived from patients in CML blast crisis would probably preclude the use of FTI as monotherapy in advanced phases of the disease. However, due to observed potentiation of imatinib FTI may find their place as supplement to treatment modalities for otherwise refractory BCR-ABL-positive cells. J Leuk Biol (Accepted)

Publication (⋆ = external co-author)

Pharmacokinetics/dynamics
R.E. Port

In cooperation with: PD Dr. Gunnar Brix, PD Dr. Michael Knopp, FS 05, DKFZ; Prof. Dr. O. Mehlis, Pediatric Hospital, University of Heidelberg; Prof. Dr. R.W. Jelliffe, University of Southern California, Los Angeles, USA
Grant: Pharmacodynamics of erythropoietin in children (DFG)

A major problem in drug therapy of cancer is the achievement of sufficiently high drug concentrations in malignant tissue for a sufficient length of time. The actual drug concentrations in human solid tumors are largely unknown. Dynamic contrast-enhanced magnetic resonance imaging (dMRI) allows one to monitor and visualize the concentration-time course of MRI contrast agents like gadopentetate in human solid tumors non-invasively and, thereby, can help understand the problems for systemically administered drug to reach and penetrate malignant tumor tissue. Population models were used to simultaneously analyze the pharmacokinetics of gadopentetate in human mammary tumors and in arterial blood and the requirement for individually monitoring the kinetics in arterial blood (“arterial input function”) in order to correctly interpret the measurements in tissue was demonstrated [1]. Pharmacokinetic population models were used to analyze the time course of the hematopoietic response to erythropoietin (EPO) treatment in children with renal anemia. A parametric method (NONMEM) was employed in preparing a non-parametric analysis (NPAG). No dependence of response on body weight was found when relating response to the absolute dose of EPO. This is in contradiction to general experience in drug dosing in children and puts into question the current practice of reducing the initial EPO dose in proportion to body weight. A gradual disappearance of non-hematopoietic EPO receptors with age could be an explanation.

Publication (⋆ = external co-author):