Unit Pharmacology of Cancer Treatment (D0200)

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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 focusses on pharmacokinetic and pharmacodynamic modelling.

Retroviral transfer of the human multidrug resistance-1 gene in human mobilized peripheral blood progenitor cells engrafting in NOD/SCID mice can confer chemoprotection


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Mobilized peripheral blood progenitor cells (PBPC) are the main source for autologous transplantation in cancer patients to support the rapid reconstitution of mature blood cells after chemotherapy. The transfer of the multidrug resistance-1 (MDR1) gene into hematopoietic stem (HSC) and progenitor cells (HPC) extends the concept of supportive care by protecting the bone marrow (BM) of cancer patients from myelotoxicity, the main adverse and often dose-limiting effect of most cytostatic drugs. In mice, transplantation of BM cells from MDR1 transgenic animals as well as transplantation of retrovirally transduced primary HPC resulted in chemoprotection in vivo. However, pilot clinical trials with murine retroviral vectors, so far have failed to demonstrate the feasibility of this gene therapy approach due to low transduction efficiencies of human HSC 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. Transplantation of human HSC into NOD/SCID mice provides a valuable in vivo assay to assess the gene transfer efficiency and the repopulating capacity of HSC subsequent to ex vivo retroviral infection, allowing optimization of infection protocols and retroviral vectors prior to human clinical trials. Using an improved FMEV-based vector, SF-MDR (SF91m3), designed for high expression in HSC and myeloid progenitor cells, we performed a series of in vitro and in vivo experiments to develop optimized methods for retrovirus-mediated gene transfer into CD34+ - selected PBPC from tumor patients and to create the basis for testing the concept of myeloprotective gene therapy.

Clinical scale human PBPC cell grafts transduced with the new retroviral vector SF91m3 that contains the human MDR1 gene were transplanted into NOD/SCID mice. We showed that, in this setting, 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 PBPC were used from normal donors during G-CSF mobilization.
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 six to seven weeks observation period. Longer observation periods are being investigated.

The median gene transfer rate to NOD/SCID mice-repopulating human cells derived from PBPC (SRCs) ranged between 9.4%-12.3%.

In this study, a median of 59% (33%-92%) of SF91m3 transduced repopulating cells contributed to the Rh123<sup>+</sup> phenotype. These data suggest that approximately one vector integration had occurred in any MDR1-transduced NOD/SCID engrafting cell with the transduction protocol used, thus limiting the risk of insertional mutagenesis as much as it is possible with retroviral vectors.

The high level of engraftment, gene transfer and gene expression allowed to study in vivo chemoprotection. We chose paclitaxel treatment (total dose 40 mg/kg), the highest dose at which all NOD/SCID mice survived in a dose-finding study until day 24 after drug administration. This dose allowed to follow human leukocytes not only in the nadir period of mouse body weight, which occurred on days four to eight after start of therapy, but also after recovery on day 15.

In the BM of chemotherapy-naive mice we found values equivalent of 0.45x10<sup>9</sup>/l to 0.81x10<sup>9</sup>/l highly P-glycoprotein expressing human white blood cells (WBC) above background levels. Despite encouraging engraftment rates of MDR1-transduced cells in recent clinical trials such high, myeloprotective levels of MDR1-transduced leukocytes have not been observed before.

After paclitaxel therapy, MDR1-gene marking and P-glycoprotein expression increased significantly. It was possible to demonstrate a significant chemoprotection of MDR1-transduced human marrow engrafting leukocytes in one cohort (P<0.05) and a similar trend in the other cohort, thus supporting the rationale for conducting clinical trials with optimized retroviral vectors containing the human MDR1 gene to prevent chemotheraphy-induced myelosuppression.

Publications (* = external co-author)


New strategies for human solid tumor suicide gene therapy using recombinant adenovirus-associated virus 2 vectors

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Adeno-associated virus 2 (AAV-2) vectors are used for clinical gene therapy of hemophilia and at this stage for preclinical cancer gene therapy. In previous experiments [1, 2], we could show a high susceptibility of some solid tumor cells for a rAAV-2 vector containing the humanized green fluorescent protein (hGFP) gene. Among a series of primary cells and eight solid tumor cell lines infected with these AAV supernatants, we observed the highest infection rates (functional titer: 200 iu/cell) in soft tissue sarcoma cells (HS1; mean: 95.97%) and in breast cancer cells (T47D; mean: 82.52%, MCF-7; mean: 85.35%), the other cell lines (1 ovarian tumor, 1 germ cell tumor, 1 osteosarcoma and 2 small cell lung cancer) were less permissive (2.82% - 11.94%), while CD34<sup>+</sup> peripheral blood progenitor cells showed the lowest transduction rates (mean: 4.39%). These data suggest that 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 prodrugs aciclovir and ganciclovir. Three new vectors were cloned containing either the TK gene, the TK and the hGFP gene connected by an IRES-sequence or a TK/eGFP fusion protein, each under control of the cytomegalovirus promoter/enhancer sequences. For all the cell lines to be used in further experiments (T47D, MCF-7, HS-1 and an additional sarcoma cell lines HT1080) dose-effect curves for both aciclovir and ganciclovir were established to determine the cytotoxicity induced by cellular kinases and the optimal therapeutic index for further experiments. The TK gene in the new vectors did not allow fluorescence-based titration. Therefore, a new titration method based on the quantitative TaqMan real-time polymerase chain reaction (qPCR) was established so that an easy, quick, safe, accurate and high-throughput titering of AAV-2 stocks with and without selectable marker genes became possible. Final requirements before testing the rAAV-2 suicide system in a NOD/SCID mouse model are in vitro cell kill experiments with the AAV-TK vectors in the two human breast cancer and the two human sarcoma cell lines which are currently being performed. The xenotransplantation tumor model for the human breast cancer and sarcoma lines has already
been established, optimized and tested in our lab for both the intraperitoneal and subcutaneous transplantation route. In proof of principle experiments we should thus be able to show the effectiveness of our AAV-TK suicide gene therapy approach in the treatment of breast cancer and sarcoma.

Publications (* = external co-author)


Experimental therapy of chronic myelogenous leukemia employing tyrosine kinase inhibition

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Increased tyrosine kinase (TK) activity of the bcr/abl oncoprotein results in a reduced apoptosis and thus prolonged survival of chronic myelogenous leukemia (CML) cells. Inhibition of the BCR-ABL tyrosine kinase pathway is a promising approach for systemic therapy of chronic myelogenous leukemia (CML) patients. STI571 (formerly known as CGP57148B), a novel 2-phenylaminopyrimidine derivative, was reported to selectively inhibit the BCR-ABL tyrosine kinase and induce apoptosis in BCR-ABL -positive cells. We and others showed that STI571 inhibits colony formation of CML progenitor cells sparing the normal progenitors [1]. The recently initiated multicenter clinical trials showed high response rates at all stages of CML. However, patients in the advanced and blast phases frequently relapse. Combination therapy employing STI571 and classical chemotherapeutic agents represents a promising concept for this group of patients.

We tested whether STI571 would act synergistically in combination with a number of cytotoxic drugs or γ-irradiation on BCR-ABL positive cells [2,3]. A tetrazolium based MTT assay was used to quantify growth inhibition after 48 hrs of exposure to cytotoxic drugs alone and in simultaneous combination with STI571. Irradiation was applied prior to 48 hrs of exposure to STI571. The agent interactions were analysed using the median-effect method of Chou and Talalay. The combination index (CI) was calculated according to the classic isobologram equation. Cytarabine, etoposide, mafosfamide, mitoxantrone and γ-irradiation proved to be clearly and significantly synergistic at growth inhibition levels over 50%.

At therapeutic concentrations no relevant toxicity of STI571 on BCR-ABL negative (HL-60, KG1a) leukemic cell lines and normal CD34+ selected peripheral blood stem cells was observed. STI571 did not potentiate the effects of cytotoxic agents in BCR-ABL negative cells. In summary, combinations of these classical chemotherapeutic agents or γ-irradiation with STI571 are expected to result in a higher therapeutic index when administered to CML patients. The results of our studies form the basis for the design of a multicenter Phase I/II clinical trial for myeloid blast crisis CML patients.

Publications (* = external co-author)


Pharmacokinetics/dynamics (D0201)

R.E. Port

In cooperation with: PD Dr. Peter Bachert, PD Dr. Gunnar Brix, PD Dr. Michael Knopp, FS 05, DKFZ; Dr. Thomas Bouillon*, Dept. of Anaesthesiology, University of Bonn; Prof. Dr. O. Mehls, Pediatric Hospital, University of Heidelberg.

One of the major problems in treating cancer with drugs is the achievement of sufficiently high drug concentrations within solid malignant tumors for a sufficient length of time. Actual drug concentrations in human tumors are largely unknown. Noninvasive measuring procedures like positron emission tomography (PET), nuclear magnetic resonance spectroscopy (MRS) in vivo and dynamic magnetic resonance imaging (dMRI) allow one to monitor absolute or relative drug concentrations in tissue. A better understanding of pharmacokinetics in solid malignant tumors will hopefully contribute to the development of more effective ways of drug treatment.

Special kinetic models, taking into account the individual arterial input function during the time when tissue concentrations are monitored, were developed for the analysis of PET data from patients with liver metastases of colorectal carcinomas [1] and of dMRI data from patients with malignant and benign mammary tumors [2]. Heterogeneity in the sense of two or three different kinetic compartments in the same tumor was found in almost all malignant mammary tumors while it was an exception in mastopathic nodules; fibroadenomas seem to range in between.

The tissue pharmacokinetics of floxuridine was monitored locally at the injection site upon intratumoral and subcutaneous injection by MRS in vivo [3]. Pharmacokinetics is more variable in malignant tumors than in subcutaneous tissue and, in tumors, always has a slow component of disposition with half-life between 30 min and several hours; this demonstrates the effect of variable and partly insufficient vascularization which is one of the major obstacles to successful drug therapy by systemic administration. Drug elimination from the site of a subcutaneous injection is invariably rapid with half-life between 6 and 9 min; the combination of subcutaneous injection with local MRS in vivo appears to be a promising tool for the in vivo testing of controlled-release drug preparations designed for interstitial application.

Population modelling was applied to the pharmacokinetics of piritramide in surgical patients [4]. Ongoing population analyses deal with the arterial pharmacokinetics of the MRI contrast agent gadopentetate and the pharmacodynamics of erythropoietin in children with renal anemia. Grants: Intratumoral pharmacokinetics of floxuridine (Deutsche Krebshilfe); Pharmacodynamics of erythropoietin in children (DFG)

Publications (* = external co-author):