Treatment with the HIV protease inhibitor nelfinavir triggers the unfolded protein response and may overcome proteasome inhibitor resistance of multiple myeloma in combination with bortezomib: a phase I trial (SAKK 65/08)

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Supplemental Matreial

Methods

Eligibility

Patients with advanced MM, acute leukemia or malignant lymphoma lacking active standard treatment options were eligible for the trial. Eligibility criteria included <5 prior chemotherapy lines, ECOG PS ≤ 2, adequate hematological (neutrophils ≥1.5x10^9/L, platelets ≥75x10^9/L, hemoglobin >80 g/L; in case of bone marrow involvement platelets ≥20x10^9/L (amended to ≥50x10^9/L) and hemoglobin >80 g/L by transfusion), hepatic and renal function (bilirubin ≤ 1.5 x ULN, ALT≤ 2.5 x ULN, creatinine clearance > 30 ml/min). Major exclusion criteria included uncontrolled, clinically relevant medical conditions, CTC grade >1 peripheral polyneuropathy, and use of strong CYP3A4 modulators. In the extension cohort of the trial, only bortezomib-resistant MM patients were eligible after at least 2 lines of systemic treatment. Per protocol developed before 2011, “bortezomib-resistant” disease was defined as “nonresponsiveness or progression to bortezomib-containing therapy, or progression < 6 months after completing such therapy”. We refer to this definition when we use “bortezomib resistance” in the current manuscript. These criteria were superseded by respective international myeloma working group (IMWG) consensus recommendations 2011 [32],

where the term “bortezomib-refractory” is being used. In agreement with IMWG criteria, we here refer to “bortezomib-refractory myeloma” as myeloma “nonresponsive while on bortezomib-containing therapy, or progressive within 60 days of last bortezomib-containing therapy” [32].

All patients gave written informed consent prior to trial inclusion, the trial was approved by the independent cantonal research ethics committees, performed in accordance with national Swiss law, ICH-GCP, and the Declaration of Helsinki, and registered (NCT01164709).

Trial design

The primary endpoint of this prospective, multicenter phase I dose escalation trial was dose limiting toxicity (DLT). Secondary endpoints included adverse events (AE), pharmakodynamic (proteasome inhibition, p-AKT and UPR)/pharmacokinetic parameters and response to trial treatment. Dose escalation was performed in a classical 3+3 design. AE were graded according to CTCAE 4.0. DLT was assessed during cycle 1 in all patients that have received at least one dose of bortezomib in combination with nelfinavir, and defined as hematologic toxicity grade 3-4 persisting for > 2 weeks, or any grade ≥ 3 non-hematological AE judged at least possibly related to nelfinavir or bortezomib, excluding self-limiting grade 3 ALT, bilirubin or metabolic changes (cholesterol, blood glucose, triglycerides), grade 3 nausea/vomiting without adequate symptomatic therapy or grade 3 neurotoxicity without dose reduction of bortezomib.

After establishing the recommended dose, the protocol was amended to treat an exploratory extension cohort of six additional patients with bortezomib-resistant myeloma at the recommended dose to assess toxicity and detect early signals of activity. Patients with bortezomib-resistance diagnosed within the last 12 months received bortezomib plus nelfinavir, while patients with bortezomib-resistance > 12 months before inclusion were treated with one cycle of bortezomib monotherapy d 1, 4, 8, 11 to confirm current bortezomib resistance per protocol, prior to the initiation of treatment with bortezomib+nelfinavir. Data for treatment regimens before and after trial treatment were collected from hospital charts.

Drug administration

Bortezomib was given at 1.3 mg/m^2 d 1, 4, 8, 11 i.v. for three cycles of 21 days. No dexamethasone was added during the dose escalation part. Nelfinavir (dose levels 1250 mg (DL0), 1875 mg (DL1) and 2500 mg (DL2)) was taken on days 1-14 as 625 mg capsules q 12 h p.o. together with a full meal. In cycle 1 only, combination treatment with bortezomib+nelfinavir was preceded by nelfinavir monotherapy on days -7 to -1 (run in phase for PK/PD assessment). Patients without disease progression after cycle 3 could continue trial therapy until completion of 7 cycles. In the extension cohort, the run-in phase of nelfinavir was omitted in all except one patient, bortezomib was allowed as either i.v. or s.c. application, and dexamethasone 8 mg prior to bortezomib administration was allowed in patients achieving less than a MR after cycle 3.

DLT and response assessment

Adverse events were recorded throughout the trial for all patients. Patients completing 3 cycles of trial treatment were eligible for objective response assessment per protocol in

the dose escalation cohort (patients 1-12), based on (IMWG) criteria [33] or standard criteria for leukemia and lymphoma [34]. In the extension cohort (patients 13-19), treatment activity was assessed by IMWG criteria, incorporating minimal response (MR) per European Blood and Marrow Transplantation criteria[35], as best serum paraprotein response during trial treatment. Response assessment was peformed centrally by the SAKK.

Pharmakokinetic and Pharmakodynamic assessment

Blood samples were collected from patients 1-12 at the following timepoints: PK1 d -7 (pre nelfinavir); PK2 day -3 (4 h (± 1h) post nelfinavir); PK3 d1 (pre nelfinavir and bortezomib); PK4 d1 (4h (± 1h) post nelfinavir and bortezomib); PK5 d2 (pre nelfinavir). PBMC and serum were prepared and frozen immediately. UPR related proteins (pIRE1, CHOP, BIP, PDI, PARP) and AKT (pAKT) were measured after SDS-PAGE and western blot from PBMC lysates by fluorescence scanning, as described [29]. Quantitative assessment of proteasome activity in PBMC was performed using fluorophore-labeled activity-based probes (ABP) [36, 22]. Statistical significance of differences was assessed by Student's t-test for paired samples. Serum levels of nelfinavir and its metabolite M8 were measured by LC-MS/MS. Population pharmacokinetic modeling of nelfinavir and M8 was performed using NONMEM version 7.2 (Supplemental material). Statistical tests were justified as appropriate by the SAKK statistics section.

Supplemental figure 1

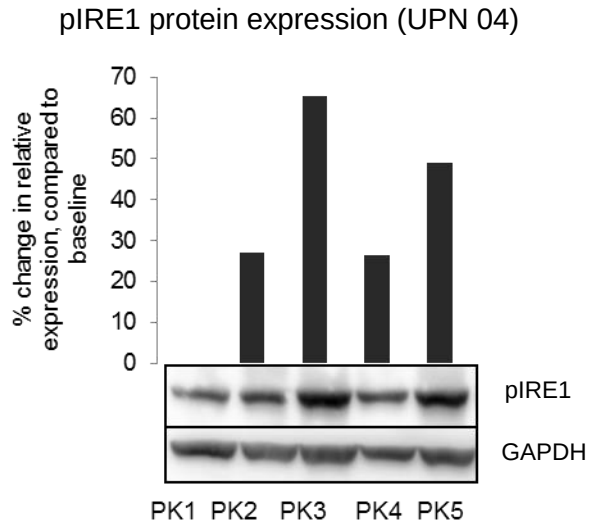

Figure legend:

Expression of pIRE1 in PBMC from a treated patient

PBMC from UPN04 collected at the indicated timepoints PK1 (baseline pre-dose), PK2 (nelfinavir 4h post-dose), PK3 (nelfinavir trough + bortezomib pre dose), PK4 (nelfinavir+bortezomib 4 h post-dose), PK5 (nelfinavir trough, 24 h post bortezomib) were probed for expression of pIRE1 protein by western blot, followed by quantitative fluorographic assessment. GAPDH served as a control. Bargraphs represent the relative increase in pIRE1 protein signal, compared to the baseline sample (PK1).

Supplemental table 1: Population pharmakokinetic model

Supplemental Table 1. Nelfinavir population pharmacokinetic parameter estimates

Parameter	Units	Final covariate model					Effect of autoinduction on drug clearance	
Nelfinavir		Estimate	RSE (%)	IIV (%)	RSE on IIV (%)	S (%)	Equation (AI for time >5 days after NLF)	RSE (%)*
CL_{NLF}	L/min	33.9	39.2	14.4	26.3	16.8	CL_{NLF} = 33.9 L/min·(1.69) ¶	41.5
CL_{M8}	L/min	1.71	26.5	26.2	37.4	13.9	CL_{M8} = 1.71 L/min·(2.20) ¶	38.2
V_{NLF}	L	33.9	37.2	NA	NA	18.9		
K_a	h^{-1}	0.15	41.2	85.2	77.3	9.6		
F_{M8}		0.01	47.2	NA	NA	13.2		
RV_{NLF}	%	52.8	36.2					
RV_{M8}	%	48.7	31.5					

RSE=relative standard error, IIV=interindividual variability, RV=residual variability, V=volume of distribution, CL=clearance, NA=not available, S=shrinkage, AI=autoinduction, NLF=nelfinavir, M8=nelfinavir active metabolite, K_a=absorption rate constant, F_{M8}=fraction of nelfinavir clearance for the formation of M8

¶ the termin within parentheses represents autoinduction that results in an increase of drug clearance for time-points >5 days after the administration of nelfinavir

* relative standard error on the autoinduction term (within parentheses)

Supplemental figure 2 (goodness-of-fit plots of nelfinavir and M8):

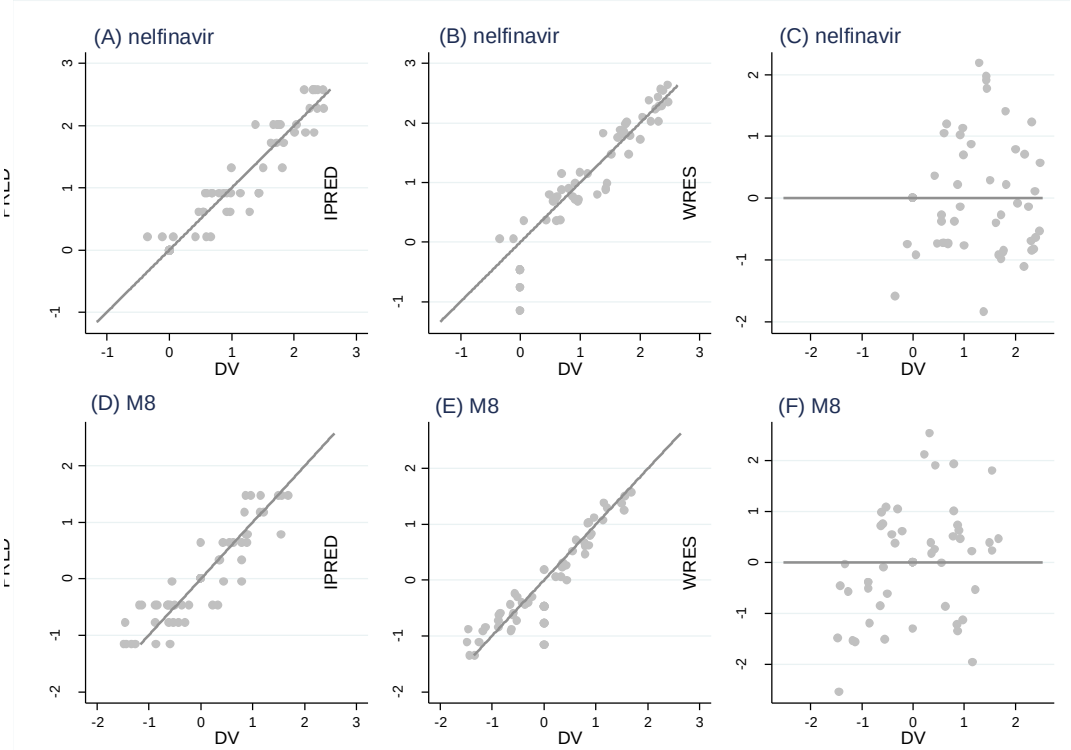

Figure Legend:

Goodness-of-fit plots of the final model for nelfinavir and M8 pharmacokinetics. Observed nelfinavir plasma concentrations (DV) versus model-predicted concentrations (PRED) (A), individual predicted concentrations (IPRED) (B) and weighted residuals (WRES) (C). Observed M8 plasma concentrations (DV) versus model-predicted concentrations (PRED) (D), individual predicted concentrations (IPRED) (E) and weighted residuals (WRES) (F).