Leukemia Research 68 (2018) 62–69

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Safety and efficacy of the CD95-ligand inhibitor asunercept in transfusion-dependent patients with low and intermediate risk MDS

Tobias Boch[a,b], Thomas Luft[c], Georgia Metzgeroth[a,b], Maximilian Mossner[b], Johann-Christoph Jann[b], Daniel Nowak[a,b], Franziska La Meir[a,b], Christiane Schumann[b], Jennifer Klemmer[c], Susanne Brendel[b], Harald Fricke[d], Claudia Kunz[d], Christel Weiß[e], Wolf-Karsten Hofmann[a,b], Florian Nolte[a,b,*]

[a] Department of Hematology and Oncology, University Hospital Mannheim, Mannheim, Germany
[b] Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[c] Department of Internal Medicine V, Heidelberg University, Heidelberg, Germany
[d] Apogenix AG, Heidelberg, Germany
[e] Department of Medical Statistics and Biomathematics, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany

ARTICLE INFO

Keywords:
Myelodysplastic syndromes
Apoptosis
Clinical trial
CD 95
Fas ligand
Erythropoiesis

ABSTRACT

In low risk MDS, increased apoptosis of erythroid progenitors mediated via CD95 (Fas) activation has been described to result in peripheral cytopenia. Blockade of the CD95 system can improve erythropoiesis in MDS. Asunercept (APG101) is a fusion protein consisting of the extracellular domain of human CD95 and the Fc domain of human IgG1 blocking the interaction between CD95 and its ligand. Here we report on results from a phase I study in 20 transfusion-dependent low and intermediate risk MDS patients treated with intravenous asunercept (EudraCT 2012-003027-37). Primary objectives were safety and tolerability as well as pharmacodynamic effects. Secondary objectives were hematologic improvement, incidence and time to leukemic progression as well as overall survival. Frequency and severity of adverse events were in range of what could be expected in a patient cohort comprising of elderly MDS patients. Two patients experienced a serious adverse event with a suspected relationship to asunercept. The incidence of disease progression was low. In the 20 patients a decrease of the transfusion need from a mean of 10,8 (± 5,1) pRBCs during the 12 weeks treatment phase to a mean of 10,0 (± 4,2) pRBCs at the end of the study was observed. In conclusion, asunercept was well tolerated and showed efficacy in transfusion-dependent low and intermediate risk MDS patients. Further clinical investigation is warranted, particularly in combination with erythropoiesis stimulating agents (ESAs).

1. Introduction

Myelodysplastic syndromes (MDS) are a group of heterogeneous oligoclonal stem cell disorders characterized by peripheral cytopenia and a risk of transformation into acute myeloid leukemia (AML). Anemia is the most frequent symptom and reflects the impaired erythroid cell maturation.

The regulation of erythropoiesis partially depends on the CD95 system. CD95 is a member of the death receptor family and among other effects initiates caspase-dependent apoptosis when activated by its ligand (CD95L). Immature CD95-positive erythroblasts undergo apoptotic death when interacting with mature erythroid precursors that express CD95L but also by interaction with erythroblasts at the same stage of differentiation [1,2].

In low risk MDS, a pro-apoptotic milieu with increased activity of apoptosis-promoting factors such as tumor necrosis factor and CD95L has been described [3–5]. The inhibition of CD95 signaling by ectopic expression of a mutated FADD decreased caspase-8 activation and inhibited apoptosis of MDS erythroid precursors [6]. Thus, inadequate activation of the CD95 system may induce excessive apoptosis in MDS erythroid cells and may contribute to impaired erythroid differentiation and anemia. Moreover, recently published results from *in vitro* studies suggest that overexpression of CD95 on erythroid precursor cells is associated with resistance to treatment with erythropoiesis stimulating agents (ESAs) [7].

ESAs have shown benefit in anemic MDS patients, particularly in those with low transfusion burden and low endogenous levels of erythropoietin [8,9]. Response rates range between only 30% and 60% and

* Corresponding author at: Universitätsklinikum Mannheim, III. Medizinische Klinik Hämatologie und Onkologie, Theodor-Kutzer-Ufer 1-3 68167 Mannheim, Germany.
E-mail address: florian.nolte@medma.uni-heidelberg.de (F. Nolte).

https://doi.org/10.1016/j.leukres.2018.03.007
Received 24 November 2017; Received in revised form 7 March 2018; Accepted 9 March 2018
Available online 10 March 2018
0145-2126/

median response duration is approximately 2 years, i.e. all patients will eventually become transfusion dependent again [8–10].

In 2016, Raimbault et al. published data from *ex vivo* studies examining the impact of CD95L inhibition by asunercept on the erythropoiesis in low risk MDS patients. Asunercept rescued erythropoiesis and increased the number of burst-forming unit-erythroid (BFU-E) progenitors. This was exclusively the case in patient samples characterized by an initially low BFU-E forming capacity, i.e. asunercept was able to rescue erythropoiesis particularly in those patients whose hematopoiesis was most severely impaired. Improved BFU-E forming capacity was associated with a decrease in apoptosis of erythroid progenitors [7].

Asunercept is a glycosylated fusion protein consisting of the extracellular domain of the human CD95 receptor and the Fc domain of human IgG1 which effectively binds to CD95L expressed on effector cells as well as to functionally active ligand in solution, blocking the interaction with CD95 and inhibiting CD95 activation.

The aforementioned effects of a CD95 blockade on the erythropoiesis in MDS in an *in vitro* study provided the scientific rationale for this clinical phase I study [7]. We investigated the safety and tolerability as well as pharmacodynamic effects of asunercept on the erythropoiesis in patients with low and intermediate risk MDS that were either resistant to ESA or had a low chance of response according to the Nordic Score [8].

2. Methods

2.1. Study design

This was a prospective, open-label, single arm phase I study investigating the safety, tolerability and pharmacodynamic effects of asunercept on erythropoiesis in transfusion-dependent MDS patients (EudraCT. 2012-003027-37). The study was performed in accordance with ICH-GCP guidelines, the Declaration of Helsinki and all relevant regulations. Asunercept was given once weekly. Following a 4-weeks screening phase, the treatment period was 12 weeks. Patients were followed for another 24 weeks. Bone marrow biopsies were performed at baseline prior to first asunercept infusion, at the end of treatment (EoT), after 12 and 24 weeks of follow-up (end-of-study visit; EoS). After the treatment period, dosing was stopped irrespective of response to treatment. The primary objectives were safety and tolerability. Secondary objectives included hematological improvement, time to leukemic progression and overall survival.

2.2. Study population

Patients had to meet the following inclusion criteria: morphologically confirmed diagnosis of MDS according to the WHO-classification with low or intermediate risk according to WPSS [11,12] either previously treated or untreated; refractoriness to ESA (as assessed after at least 8 weeks of treatment with ESA) or with a low probability of response (score +1 or less according to Hellström-Lindberg [8]); red blood cell (pRBC) transfusion need of at least 4 units during the last 8 weeks prior inclusion. Main exclusion criteria were medullary blast count ≥5%, secondary or treatment-related MDS, CMML, high risk karyotype per WPSS, parallel treatment with ESA or any other experimental therapy, previous chemotherapy (including 5-azacitidine), previous treatment with lenalidomide and patients who were scheduled for allogeneic stem cell transplant within the following 6 months. The trial was conducted at the University Hospital Mannheim and the University Hospital Heidelberg.

2.3. Dose justification and treatment schedule

Claessens et al. described that a complete blockade of the CD95 system restored erythropoiesis in MDS patients in an *in vitro* system with ectopic expression of a mutated FADD [6]. Therefore, dosing of asunercept was based on the objective to achieve a pharmacological blockade of CD95 activation. In a previous study with healthy volunteers, a > 95% CD95L occupancy was maintained at the trough level using 400 mg of asunercept once weekly, (approximately 30 μg/mL serum) [13]. However, in *in vitro* experiments using primary $CD34^+$ cells from patients with low risk MDS, it was found that a maximum proliferative capacity of BFU-Es was achieved at asunercept concentrations of 10 μg/mL and further increases did not result in a better BFU-E forming capacity. PK modeling was carried out and the simulation for the dose level of 100 mg/weekly showed a minimum serum concentration of at least 10 μg/mL at steady state. Based on these findings, it was decided to proceed with 100 mg as per amendment 02 (dated 18 July 2013) after 6 patients had received 400 mg. Asunercept was administered intravenously via a 30–60-min infusion. 40 mL of asunercept solution was added to 250 mL of physiologic salt solution for the 400-mg-dose, i.e. the total volume infused to each patient was 290 mL. For the 100-mg-dose, 5 mL APG101 solution were added to 250 mL NaCl resulting in 255 mL total volume. No premedication was administered routinely.

2.4. Safety assessments

The primary objectives safety and tolerability were assessed at regular intervals by documentation of adverse events (AE), physical examinations, laboratory assessments (hematologic, serum chemistry and serological measurements at the local laboratories), electrocardiography and ultrasound evaluations. Regarding leukemic progression, microscopic differential blood counts at regular intervals and marrow evaluations including cytomorphology, flow cytometry and cytogenetics were performed at screening, EoT (week 13), week 25 and EoS (week 37). AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 19.0 and graded as mild, moderate or severe. Treatment-emergent adverse events (TEAEs) were defined as AEs occurring or worsening after start of study treatment. TEAEs with suspected relationship to study drug were defined as “definitely”, “probably” or “possibly” or “unknown” related to study drug per the investigator's assessment. Moreover, incidence and time to leukemic progression as well as overall survival at 9 months were evaluated.

2.5. Efficacy assessment

The evaluation of hematopoietic improvement was a secondary objective in this trial. All patients enrolled into this trial were transfusion-dependent. In a few patients, the complete documentation of the pRBCs transfused prior to study entry was not completely available leading to possible underestimation of the pre-treatment transfusion need. Therefore, in the statistical analysis plan (SAP, version 23 March 2016) transfusions need was compared between the treatment phase and the first and second 12-weeks post-treatment periods. Moreover, since the duration of each period differed slightly between the patients, transfusion need was calculated by the number of pRBCs adjusted to the individual number of days per period. For better clinical utility and confirmability, we provide within this manuscript the absolute numbers of transfused pRBC per pre-planned periods (treatment phase: day 1–84; 1. post-treatment phase: day 85–167; 2. post-treatment phase: day 168–252) as post-hoc analyses. In addition, IWG response criteria [14] comparing the transfusion burden during 8 weeks prior study enrollment to the 8 weeks post-treatment period were also applied and reported as post-hoc analyses.

2.6. Statistical analysis

The sample size was set without test power considerations. A total of 18 MDS-patients with low and intermediate risk features per WPSS