



Individualized long-term outcomes in blood phenylalanine concentrations and dietary phenylalanine tolerance in 11 patients with primary phenylalanine hydroxylase (PAH) deficiency treated with Sapropterin-dihydrochloride[☆]

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ABSTRACT

We analyzed long-term sustainability of improved blood Phenylalanine (Phe) control and changes to dietary Phe tolerance in 11 patients (1 month to 16 years), with various forms of primary PAH deficiency (classic, moderate, severe phenylketonuria [PKU], mild hyperphenylalaninemia [HPA]), who were treated with 15–20 mg/kg/d Sapropterin-dihydrochloride during a period of 13–44 months.

7/11 patients had a sustainable, significant reduction of baseline blood Phe concentrations and 6 of them also had an increase in mg/kg/day Phe tolerance. In 2 patients with mild HPA, blood Phe concentrations remained in the physiologic range even after a 22 and 36% increase in mg/kg/day Phe tolerance and an achieved Phe intake at 105% and 268% of the dietary reference intake (DRI) for protein. 2 of these responders had classic PKU.

1 patient with mild HPA who started treatment at 2 months of life, had a significant and sustainable reduction in pretreatment blood Phe concentrations, but no increase in the mg/kg/day Phe tolerance. An increase in Phe tolerance could only be demonstrated when expressing the patient's daily Phe tolerance with the DRI for protein showing an increase from 58% at baseline to 78% of normal DRI at the end of the observation.

Long-term follow-up of patients with an initial response to treatment with Sapropterin is essential to determine clinically meaningful outcomes. Phenylalanine tolerance should be expressed in mg/kg/day and/or % of normal DRI to differentiate medical therapy related from physiologic growth related increase in daily Phe intake.

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1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive deficiency of phenylalanine hydroxylase (PAH) resulting in an accumulation of phenylalanine (Phe) in blood and in the brain. Cognitive/behavioral deficiency is prevented by early institution of a Phe restricted medical nutrition therapy [1,2]. However, recommended blood Phe concentrations [3,4] are difficult to maintain over the long-term. Despite early diagnosis via newborn-screening programs, the prevalence of neuropsychiatric (ADHD, anxiety, depression) and executive

functioning problems is high in this patient population [5,6]. Poor blood Phe control correlates with the severity of the PKU [7], and is of particular concern in adolescents and adults [8–11] and in children from families with insufficient psychosocial resources [12].

Sapropterin dihydrochloride (Sapropterin, Kuvan®) is a PAH cofactor [13] with the ability to reduce blood Phe concentration and to increase dietary Phe intake. The effect is believed to be due to Sapropterin's chaperon function and its ability to increase the residual enzyme activity. It is estimated that about 30–50% of patients respond to therapy with Sapropterin [14–18] with a decrease in blood Phe concentrations. Patients with milder forms of PKU and higher residual PAH activity seem to be particularly responsive to Sapropterin, but patients with severe/classic PKU may also respond [14,19].

Responsiveness to Sapropterin has been defined as 30% reduction of blood Phe concentrations after an initial Sapropterin trial in a variety of study protocols [17,20–24]. Recently less pronounced reductions have also been accepted [25]. Some authors have shown that a reduction

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of blood Phe concentrations during the first 24 h of Sapropterin administration is predictive of a long-term response [26]; others suggest a one month trial to determine a response [27].

Despite an increasing number of outcome studies [18,28–32] information about long-term sustainability of reduced and absolute blood Phe concentrations and dietary Phe tolerance achieved upon treatment with Sapropterin is still limited.

Here we report long-term outcomes of blood Phe concentrations and dietary Phe tolerance in 11 patients in response to therapy with Sapropterin.

2. Patients & methods

2.1. Study protocol

We performed a chart review (Institutional Ethics Review board approval # H12-03598) in 11 patients diagnosed with primary PAH deficiency via newborn screening who had shown a >30% reduction of blood Phe concentrations after one month of treatment with 20 mg/kg/day Sapropterin. The initial response was determined by comparing mean blood Phe concentrations after 1 month of treatment with 1-month pretreatment values. To determine long-term sustainability we investigated whether the initial blood Phe reduction was sustainable, we compared mean 6-month treatment- with 6 month-pretreatment blood Phe concentrations. To determine whether treatment with Sapropterin resulted in a sustainable improvement of Phe tolerance, we compared the dietary Phe intake during the last 3 months of the entire observation period with the 6-month pretreatment Phe intake. The data were collected retrospectively on clinical observations made between 2009 and 2013.

2.2. Patient characteristics

For classification of the severity of the PKU phenotype, we applied generally used criteria based on pretreatment blood Phe concentrations: Classic PKU: >20 mg/dl (>1200 $\mu\text{mol/l}$); moderate PKU: 15–20 mg/dl (900–1200 $\mu\text{mol/l}$); mild PKU: 15–20 mg/dl (600–900 $\mu\text{mol/l}$); mild Hyperphenylalaninemia (mild HPA): <10 mg/dl (<600 $\mu\text{mol/l}$); non-PKU HPA: 2–6 mg/dl (120–360 $\mu\text{mol/l}$) [33].

PAH genotype was not available in the patients included in this study, as mutation analysis historically has not been part of the diagnostic confirmation.

2.3. Standard treatment

Patients were treated according to a standard medical nutrition therapy protocol including a Phe restricted diet, medical Phe-free aminoacid formula and low protein foods with the aim to maintain therapeutic blood Phe concentrations of 2–6 mg/dl (120–360 $\mu\text{mol/l}$) and allowing for blood Phe concentrations of up to 10 mg/dl (600 $\mu\text{mol/l}$) in patients older than 12 years.

2.4. Sapropterin treatment

Sapropterin was administered orally once daily with a main meal. Initially all patients were started on 20 mg/kg/day. With increasing weight, dosages down to 15 mg/kg/day were allowed until new adjustments were made.

6/11 patients (P5, P6, P7, P8, P9, P10) received Sapropterin when enrolled in PKU-015 (Biomarin Pharmaceutical Inc), an ongoing open label trial to determine the safety and efficacy of Sapropterin dihydrochloride on blood Phe concentrations and neurocognitive outcomes in children with PKU between 0 and 6 years [34].

2/11 patients (P1, P3) were studied after completion of PKU-016 (Biomarin Pharmaceutical Inc), a randomized controlled trial to determine the safety and effect of Sapropterin on neuropsychiatric symptoms

in PKU patients [35]. Because PKU-016 study started with a 13 week-randomized, double blinded treatment period, treatment start for the patients included here, was set arbitrarily at the beginning of the consecutive 13 week-open label phase. 3/11 patients (P2, P4, P11) received Sapropterin as part of clinical care. P11, whose treatment started at 1 month of age, only had a 1 month pre-treatment baseline mean Phe concentration.

2.5. Blood Phe measurements

The number of blood Phe measurements was defined according to the clinic's standard protocol for monitoring dietary control: blood Phe concentrations being measured with a minimum frequency of twice weekly in children younger than 6 months, once weekly in children between 6 months and 18 months, once every 2 weeks in children between 18 months and 12 years, and once monthly after completion of the 12th year of life. During the 6 month-treatment period, blood Phe concentrations were measured in weekly intervals during the first month of treatment with Sapropterin and afterwards according to the clinic's standard protocol.

Blood samples were collected after a 3–6 hour fasting period in children younger than 2 years and after a 6–10 hour overnight fast in older children. Blood Phe concentrations were determined in dried blood spots obtained during regular home-based monitoring, or in plasma from blood samples obtained during a clinic visit. Dry blood spot and plasma amino acid analysis was performed using liquid chromatography–mass spectrometry technology and an amino acid analyzer based on ion exchange chromatography, respectively.

As in our institution blood Phe concentrations are given in mg per deciliter (mg/dl) we will use this unit throughout the text. The conversion factor of mg/dl to $\mu\text{mol/l}$ is 60.4.

2.6. Data analysis

We performed paired *t*-test comparing intra-individual mean blood Phe concentrations before and during treatment. Statistical significance was defined as $p < 0.05$.

2.7. Dietary Phe tolerance

To evaluate the long-term effects of treatment with Sapropterin on dietary Phe tolerance, we assessed dietary Phe intake achieved at the last 3 months of the individual observation treatment period and compared this value with 6-month pretreatment tolerance. We used prescribed dietary Phe intake as an indicator as diet records containing actual dietary Phe intake data was not systematically available.

We determined dietary Phe tolerance in 3 ways: total daily Phe intake expressed as milligrams Phe per day (mg/d); Phe intake per body weight expressed as milligrams per kilo per day (mg/kg/day); and DRI related Phe intake expressed as % normal DRI, comparing the patient's daily Phe intake with the Dietary Reference Intake (DRI) for age related normal peers. Phe norms were derived from the age specific norms of daily protein intake, multiplied by 47, given that 1 g protein = 47 mg Phe. Dietary Reference Intakes (DRIs) of Phe consumption were calculated from age specific recommended dietary allowance (RDA) tables from the Institute of Medicine DRI report [36].

3. Results

3.1. Patient characteristics

7 males and 4 females between 1 month and 16 years of age (median 5 years) were treated for 13–44 months (median 26). At the end of the individual treatment period, patients' age ranged from 2 to 18 years (median 9). 5 patients had classic, 1 had moderate, 1 had mild PKU, and 4 patients had mild HPA. 1 of the 4 patients with mild HPA (P8)

Table 1
Changes in blood Phenylalanine concentrations and dietary Phenylalanine tolerance in response to treatment with Sapropterin dihydrochloride in 11 patients with primary Phenylalanine hydroxylase deficiency (Phenylketonuria/Hyperphenylalaninemia).

Pt #, Gender (m/f)	Untreated blood Phe concentrations (mg/dl) & severity [33]	Age at treatment start (years/months)	Total treatment period (months)	Mean (+/–SD) blood Phe concentrations			% change in blood Phe concentrations		Total daily Phe tolerance (mg/day)		Mg/kg Phe tolerance (mg/kg/d)			Phe intake (% DRI)		
				Baseline (minus 6 months)	Treatment (plus 6 months)	Treatment (last 6 months)	(plus 6 months) vs (minus 6 months)	(plus 6 months) vs (last 6 months)	Baseline (minus 6 months)	Treatment (last 3 months)	Baseline (minus 6 months)	Treatment (last 3 months)	% increase total Phe tolerance	% increase mg/kg Phe tolerance	Baseline (minus 6 months)	Treatment (last 3 months)
1(m)	83.0 (c) ^a	16 y	16	5.83 (±0.63)	2.57 (±1.11)	2.39 (±0.83) ^b	–56	–59	550	1200	6.9	14.7	118	113	17	37
2(m) ¹	38.6 (c)	11 y	24	3.30 (±3.06)	3.53 (±3.19)	3.03 (±1.53)	7	–8	473	508	8.6	7.9	8	–9	19	20
3(m)	7.8 (mHPA)	9 y	15	3.74 (±0.61)	2.00 (±0.33) ^b	1.70 (±0.22) ^b	–47	–55	1475	1880	38.4	47.0	27	22	86	105
4(m)	20.4 (c)	8 y	22	4.61 (±3.13)	2.36 (±1.17)	3.88 (±0.92)	–49	–16	478	870	15.0	22.7	82	51	34	51
5(m) ¹	30.0 (c)	6 y	43	5.56 (±1.93)	6.44 (±4.32)	5.81 (±2.47)	16	4	250	360	10.2	9.9	44	–3	23	22
6(m)	20.3 (c)	5 y	42	4.99 (±2.85)	4.13 (±1.85)	3.69 (±3.45)	–17	–26	390	460	12.7	8.7	18	–31	28	19
7(f)	19.0 (mo)	5 y	44	5.42 (±0.61)	3.62 (±1.29) ^b	3.35 (±0.55) ^b	–33	–38	425	700	19.2	21.9	65	14	43	49
8(f)	4.7 (nP-HPA)-(mHPA)	2y	13	3.13 (±0.85)	1.91 (±0.67) ^b	1.87 (±0.44) ^b	–39	–40	1200	2131	97.6	132.4	78	36	197	268
9(f)	12 (m)	18 mo	43	3.41 (±1.37)	2.07 (±0.55) ^b	2.33 (±0.40) ^b	–39	–32	205	640	21.1	34.6	212	64	43	77
10(m) ²	9.7 (mHPA)	13 mo	42	3.20 (±1.58)	2.95 (±0.63)	2.59 (±0.32)	–8	–19	360	645	33.3	32.4	79	–3	67	73
11(f) ²	7.3 (mHPA)	1 mo ^c	26	6.59 (±2.15) ^c	2.10 (±0.42) ^b	3.40 (±0.61) ^b	–68	–48	214	435	41.2	38.5	103	–6	58	78

¹P2, P5 and ²P10, P11 are siblings.

(c) = classic/severe PKU; (mo) = moderate PKU; (m) = mild PKU; mHPA) = mild HPA; (nP-HPA) = non-PKU HPA.

^a Premature newborn (28 wk); Phe concentrations obtained while on total parenteral nutrition.

^b Paired *t*-test comparing mean blood Phe concentrations before and during treatment is statistically significant (*p* < 0.05).

^c 1 month-baseline pre-treatment Phe concentration (6 month-value was not available due to young age of patient).

had non-PKU HPA with blood Phe concentrations below (6 mg/dl; 360 $\mu\text{mol/l}$) until introduction of solid foods at the age of 1 year, when blood Phe concentrations rose to the 6–10 mg/dl (360–600 $\mu\text{mol/l}$) range consistent with mild HPA.

An overview of patients and changes in their mean blood Phe concentrations and dietary Phe tolerance during pretreatment and treatment periods is shown in Table 1.

3.2. Blood Phe concentrations

1 month pretreatment and 1 month treatment mean blood Phe concentrations ranged between 3.08 mg/dl (186.5 $\mu\text{mol/l}$) and 7.54 mg/dl (456.5 $\mu\text{mol/l}$) (median 4.62 mg/dl; 280 $\mu\text{mol/l}$) and 1.61 and 2.72 mg/dl (97.5 and 164.7 $\mu\text{mol/l}$) (median 2.44 mg/dl; 147.7 $\mu\text{mol/l}$) respectively, representing a range of blood Phe reduction from minus 30% to minus 69% (median 49%).

After 6 months of treatment, 7 of the 11 patients (P1, P3, P4, P7, P8, P9, P11) continued having >30% mean blood Phe reduction compared to 6 month-pretreatment values (range: minus 33–minus 68%, median: minus 47%).

6 out of these 7 patients (P1, P3, P4, P7, P8, P9) had an additional increase in mg/kg/day Phe tolerance. Their individual mean blood Phe concentrations ranged from 1.70 to 3.40 mg/dl (102.9 to 205.8 $\mu\text{mol/l}$) (median 2.36 mg/dl; 142.9 $\mu\text{mol/l}$) and were significantly ($p < 0.05$) lower compared to 6-month pretreatment values.

4 out of 11 patients (P2, P5, P6, P10) did not maintain their initial >30% blood Phe reduction after 6 months of treatment.

At the end of the observation period (last 6 months of treatment), patients had blood Phe concentrations between 1.7 and 5.81 mg/dl (102.9 and 351.7 $\mu\text{mol/l}$) (median 3.03 mg/dl; 183.4 $\mu\text{mol/l}$).

3.3. Dietary Phe tolerance (Fig. 1)

At the end of the individual treatment period, all patients had an increase in total mg/day dietary Phe tolerance (range 8%–212%, median 78%). 6 patients (P1, P3, P4, P7, P8, P9) also had an increase in mg/kg/day Phe intake ranging from 14% to 113% (median 43.5%). The maximum effect was observed in P1 with an increase of mg/kg/day Phe tolerance by 113% (from 6.9 mg/kg/day to 14.7 mg/kg/day).

We also compared the patients' daily Phe intake with the DRI for protein intake as a surrogate for dietary Phe needs. Patients' dietary Phe intake ranged between 17 and 197% (median 43%) of DRI for age at pretreatment baseline and between 19 and 268% (median 51%) at the end of the treatment period. P8 (non-PKU HPA/mild HPA) with a pretreatment baseline dietary Phe intake at 197% DRI achieved a further increment up to 268% DRI with treatment. The youngest patient who started treatment at 2 months (P11 with mild HPA) had approximately the same mg/kg/day Phe tolerance after 42 and 26 months of treatment compared to their baseline, while her DRI increased from 58% to 78%.

4. Discussion

In this individual case analysis we found a range of response patterns to treatment with Sapropterin dihydrochloride. The majority of our

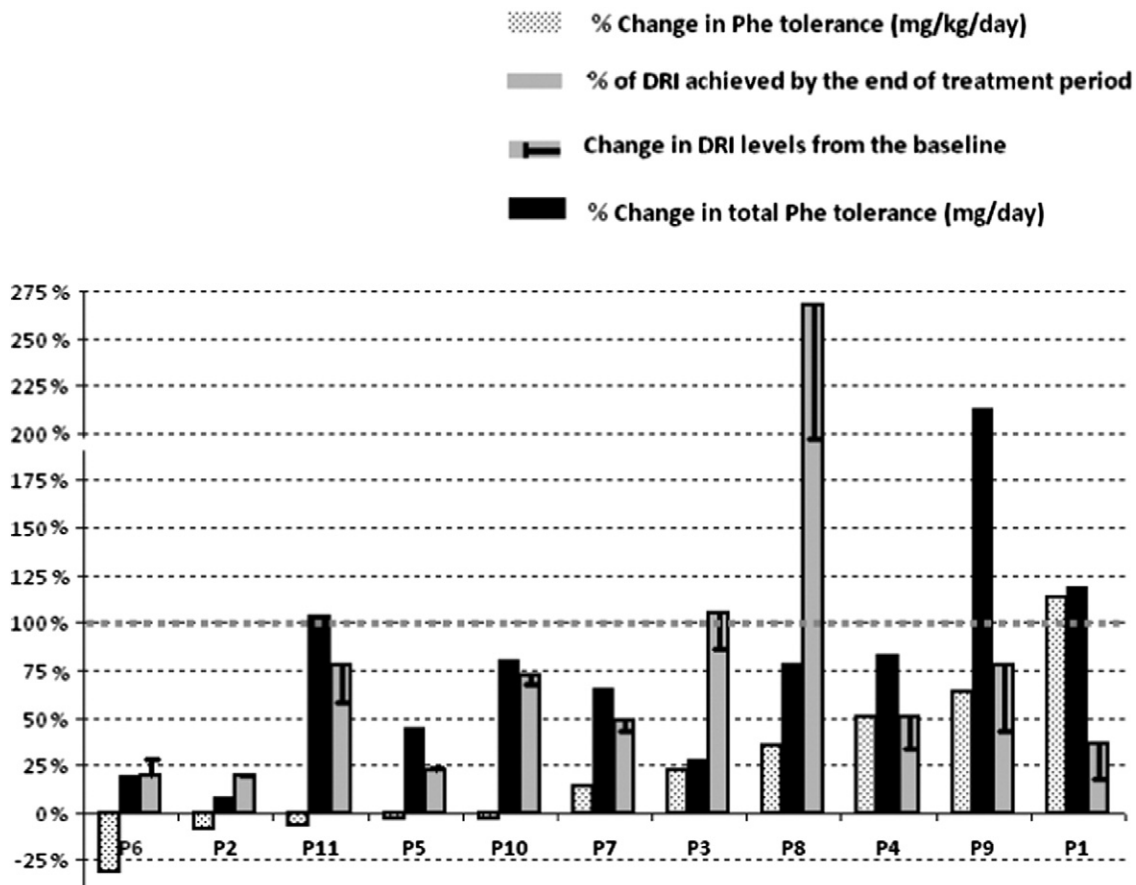


Fig. 1. Changes in dietary Phenylalanine tolerance expressed as total intake (mg Phe per day), mg Phe per kilo body weight per day and % of age related dietary reference intake (DRI) at baseline (6 months pretreatment) and the end (last 3 months) of treatment with Sapropterin in 11 patients with Phenylketonuria/Hyperphenylalaninemia.

patients had a sustainable reduction of blood Phe concentrations combined with an increase in mg/kg/day Phe tolerance.

In P3 and P8 with mild HPA, blood Phe concentrations remained in the upper physiologic range even after a 22% and 36% increase in mg/kg/day Phe intake and an achieved Phe tolerance at 105% and 268% of the DRI. Blood Phe concentrations in the physiologic range are usually not achieved in patients with PKU, even upon most arduous dietary Phe restrictions. As shown here, treatment with Sapropterin potentially allows normalization of the biochemical phenotype, particularly in patients with milder forms of PKU.

Patients with severe PKU generally are less responsive as their mutations obviously are not rescued by Sapropterin's function [32,37–39]. In our study, 5 out of 11 individuals (P1, P2, P4, P5, P6) had classic PKU according to diagnostic blood Phe concentrations above 20 mg/dl [33]. 3 of them (P2,P5,P6) did not show a long-term sustainability of initial reduction of blood Phe concentrations. While all 5 patients had some degree of an increase in total dietary Phe tolerance during the time periods studied, only in P1 and P4 this increase was also reflected in mg/kg/day and DRI related Phe tolerance. Their comparably high Phe tolerance documented during early childhood (28 and 26 mg/kg/day at 2–3 years of age) suggests a milder phenotype with some degree of residual PAH activity whereas P2, P5 and P6 had a Phe tolerance of 19–20 mg/kg/day at the same age (data not shown). P1's extremely high diagnostic blood Phe level at 83 mg/dl (5025 $\mu\text{mol/l}$) was likely further elevated due to his 28 week premature birth and parenteral nutrition at the time of diagnosis.

Various aspects deserve attention when deciding whether and to what extent an observed change in dietary Phe tolerance is the result of a Sapropterin induced response. Due to the natural, growth related augmentation in nutritional demands all children with PKU experience an increase in the total daily Phe intake with increasing age. Therefore a mg/kg/day Phe intake and comparison of the Phe intake with the age matched protein DRI (expressed in % of normal DRI) help to discriminate a "true" response from a growth related change. While all 11 patients had an increase in total daily Phe tolerance, only 6 (P1, P3, P4, P7, P8, P9) also showed an increase in mg/kg/day Phe tolerance. In very young children determination of a "true" response is even more challenging. P11 was treated with Sapropterin since the age of 2 months. After 26 months, her mg/kg/day Phe tolerance was decreased by minus 6%. Due to rapidly changing metabolic demands there is a significant natural decline in Phe tolerance during the first 2 years of life [40], manuscript in preparation]. Thus the comparatively mild decline in mg/kg/day Phe tolerance still indicates a treatment response in this patient. Alternatively, this patient shows an increment of the age related DRI from 58% of at baseline to 78% at age 2 years. Thus in cases with otherwise ambiguous response patterns, comparison of Phe intake with the age related DRI could serve as an additional tool to document an improvement. In children who have a significant change in their body mass index (BMI) (e.g. if they develop obesity during the observation period), a BMI adjusted Phe tolerance might be an appropriate measure to determine therapy-induced response. We did not use this measure in our patients as they all remained on their individual BMI percentiles during the period observed in this study.

The following limitations must be considered in the interpretation of our results: Phe tolerance was determined based on the goal to keep patients' blood Phe concentrations within the lower (2–4 mg/dl; 120–240 $\mu\text{mol/l}$) therapeutic range and further increase in Phe intake might have been possible if blood Phe levels in the higher therapeutic range (up to 6 mg/dl; 360 $\mu\text{mol/l}$) had been targeted. Another limitation is that Phe tolerance was calculated based on clinic dietitian prescribed dietary Phe goals only, whereas systematically collected actual Phe intake data analyzed from patients' diet records were not available from all patients. However, we are confident that prescribed Phe intake was similar to the actual intake, given that all patients included in this study have a history of very good compliance. Finally, availability of genotypic information might have helped to early discriminate

responders from non-responders and provided better explanations of the observed patterns.

The annual cost for Sapropterin dihydrochloride (Kuvan®) treatment is in the expensive-drug-for-rare-disease range, making a critical analysis of long-term outcomes mandatory. We suggest long-term monitoring of blood Phe concentrations to confirm sustainability of initial improvements as well as a multidimensional expression of dietary Phe tolerance to discriminate therapy-induced response from natural, growth induced changes. Recently established evidence of safety and effectiveness of Sapropterin in very young children [41,42] is in favor of starting treatment as early as possible. Integrating neuropsychological, psychosocial [43] quality of life [44] and individual goal achievements will help to further clarify what are meaningful outcomes for all involved stakeholders, including patients, health care providers, government and private payers and pharmaceutical companies.

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