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# Evaluation of a pediatric liquid formulation to improve 6-mercaptopurine therapy in children

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ABSTRACT

*Background:* 6-mercaptopurine (6-MP), a key drug for treatment of acute lymphoblastic leukemia (ALL), has until recently had no adequate formulation for pediatric patients. Several approaches have been taken but the only oral paraben-free 6-MP liquid formulation named Loulla was developed and evaluated in the target population. Preclinical and clinical evaluations were performed according to a Pediatric Investigation Plan, in order to apply for a Pediatric Use Marketing Authorization.

*Methods:* The pre-clinical study assessed the maximum tolerated dosage-volume and evaluated local mucosal toxicity of 28 daily administrations in treated compared to controls gold hamsters. The multi-centre clinical study was single-dose, open-label, crossover trial, conducted in 15 ALL children during maintenance therapy. The bioavailability and palatability of a single 50 mg fixed dose of Loulla compared to 50 mg registered tablets were evaluated in a random order on two consecutive days. Seven blood samples over 9 h were obtained each day to determine 6-MP pharmacokinetic parameters, including Tmax, Cmax, $AUC_{0-9}$ and $AUC_{0-\infty}$. A questionnaire adapted to children testing Loulla palatability and preference for either Loulla or the usual 6-MP tablet was completed. Occurrence of adverse events was determined at study visits by vital sign measurements, patient's spontaneous reporting, investigator's questioning and clinical examination.

*Results:* The preclinical study in gold hamsters showed that dosage–volume of 75 mg/kg/day was well tolerated. The relative bioavailability of liquid Loulla formulation compared to the reference presentation is 76% for $AUC_{0-9}$ and $AUC_{0-\infty}$ and 80% for Cmax. The taste of Loulla and the mouth feeling after ingestion compare favorably to the tablet. No adverse event occurred.

*Conclusion:* Pharmacokinetic, palatability and safety data support the use of Loulla in children.



## 1. Introduction

A cornerstone of successful acute lymphoblastic leukemia (ALL) treatment is attributed to an adequate 6-mercaptopurine (6-MP)/methotrexate maintenance therapy. (Schmiegelow et al., 2014) 6-MP is a purine antimetabolite which is converted into two major intracellular active metabolites. The synthetic pathway of purinergic nucleotides leads to 6-thioguanine nucleotides (6-TGN) and their cytotoxic effects result from 6-TGN incorporation into nucleic acids. Moreover, the thiopurine S-methyltransferase (TPMT) acts directly on 6-MP and also on intermediate metabolites leading to methylated derivates (6-MMPN) some of which inhibit de novo purine nucleotides synthesis. (Adam de Beaumais and Jacqz-Aigrain, 2012) 6-MP has highly variable pharmacokinetic characteristics. (Balis et al., 1998) Such variability is

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primarily attributed to incomplete and variable absorption and to the genetic TPMT polymorphism which influences the balance between 6-TGN and 6-MMPN and consequently the risk of side effects as well as treatment failure.

Daily 6-MP dosage for ALL children ranges from 50 to 75 mg/m$^2$/day during the two years of maintenance therapy. (Schmiegelow et al., 2014) Until recently, only 6-MP tablets of 50 mg were available and did not address the need of dosing flexibility. Children generally require lower daily doses. Mothers, children and health professionals have to break or even grind the tablet, take a fraction of it, and reformulate the drug for children unable to swallow tablets. (Lilleyman and Lennard, 1996; Conroy et al., 2003) This unlicenced use is at risk of medication errors subsequently impacting safety and efficacy. (Breitkreutz et al., 2007) It also carries a risk of poor compliance due to difficulties in preparing the adapted individual doses and can affect long term survival.

Experts have defined as a priority to develop adequate 6-MP formulations for children in agreement with the need officially mentioned by the regulatory authorities for unlicenced and off label medicine in children. (Schirm et al., 1992; Blumer, 1999; Nahata, 1999) Different approaches for alternative formulations were considered but none were evaluated in children.

Only for Children Pharmaceuticals (O4CP) developed an oral suspension of 6-MP appropriated to young patients (named Loulla) and submitted the development plan for a scientific advice at the Committee for Medicinal Products for Human Use (CHMP). The CHMP advised for the clinical trial design and recommended to investigate local tolerance and a pre-clinical study. Accordingly, the project was submitted and selected by the European Commission under the 7th Framework Programme for Research and Development. Both preclinical and clinical studies were conducted in order to fulfill regulatory requests by providing data on local tolerance and comparing relative bioavailability and palatability of Loulla to the reference 6-MP adult tablets used in children.

## 2. Materials and methods

### 2.1. Treatment formulations

#### 2.1.1. Reference treatment formulation

The reference treatment was the registered Purinethol® adult tablets from GlaxoSmithKline: a bottle containing 25 tablets of Purinethol® 50 mg.

#### 2.1.2. Investigational product formulation named Loulla

O4CP has developed a device named "The Shaker" (Fig. 1A) adapted for young children, with the use of a patented packaging (patent number: EP09306161-2009-11-30). The sealed child-resistant cap containing the active compound and the amber transparent double neck bottle containing the liquid vehicle are composed of high-density polyethylene (Eraclene® MR80).

The cap is a reservoir for extemporaneous system containing six fast dissolving tablets with 10 mg of 6-MP per tablet. Tablets were manufactured by MEDICE Arzneimittel PütterGmbH & Co. KG (Deutschland) according a standard procedure for preparation of solid oral dosage forms. Their appearance was plane light yellow tablets, the diameter was between 4.9 and 5.1 mm and the height was between 1.7 and 2.3 mm. Physicochemical properties (uniformity mass; average weight 50 mg; breaking strength; friability <1%; disintegration time <1 min; dissolution tests; mercaptopurine identity and assay; related substances and microbial quality) were in accordance with European pharmacopoeia.


**Fig. 1.** The Loulla "Shaker".

The 6 mL of the liquid vehicle were in the bottle and were manufactured by the Laboratory Philippe Davioud (France) according European pharmacopoeia for aqueous preparation for oral use. It appeared clear, colourless solution and free of visible particles and pH (20 °C), relative density (20 °C), extractable volume, uniformity of mass, Sodium Benzoate identity and assay and microbial quality were also conform.

Reconstitution was extemporaneous. Users pushed down and turned the childproof cap leading to deliver the 6 MEDICE 10 mg tablets into 6 ml admixture solution, after they shaked vigorously the solution and then dispensed the drug using a graduated dosing syringe of bass-density polyethylene of 5 mL (CE marked). The final concentration was 10 mg 6-MP per mL. The very fast disintegration of tablets about 9 s in average, dissolution behaviour and good stability of the thus obtained suspension long enough to maintain until administration were optimal. No particles have been observed in the freshly prepared suspension. The final oral suspension was also in accordance with European pharmacopoeia for aqueous preparation for oral use.

Long-term stability of the whole of this presentation was validated for at least 12 months.

No excipients of 6-MP tablets or liquid vehicle is contraindicated according to EMA guidelines for a pediatric use.(Table 1) (Anon, na-a; Anon, na-b) In this study, the product was not presented in its final packaging.

### 2.2. Studies design

#### 2.2.1. Preclinical studies

O4CP selected the gold hamster model approved in France for studying the mucosal toxicity for cosmetic products. The study plan was based from EMA and French guidelines. (Anon, na-c; Légifrance, 1992).

For each study, the liquid formulation was daily prepared: 6-MP fast dissolving tablets were merged to the liquid vehicle in glass container for a final oral suspension of 10 mg/ml of 6-MP and remained under magnetic agitation throughout the treatment administration to the 48 hamsters.

*2.2.1.1. Pre-clinical study 1.* The first investigation evaluated a maximum tolerated dosage-volume study with an initial dose corresponding at the maximum volume which can be administered (limited by the animals' cheek pouch sizes of 0.3 mL each) and the rat median lethal dose. Loulla was administered daily for seven days by deposition of 75 mg/kg/day under a dosage volume of 0.6 mL/day in the two cheek pouches of six gold hamsters (3 males and 3 females). A control group of 6 animals received the liquid vehicle.

Animals were checked daily for adverse clinical signs. Body weight was recorded on days 0, 1, 4 and 8. At the end of the treatment period, animals were sacrificed and subjected at a macroscopic examination.

*2.2.1.2. Pre-clinical study 2.* Two groups (treated and control groups) of 16 gold hamsters (8 males and 8 females) were tested. The treated group received Loulla daily for 28 days by deposition of the dose of 75 mg/kg/day under a dosage-volume of 0.6 mL/animal/day in the cheek pouches. In the treated group, Loulla administration was followed by a rinsing to evaluate whether this procedure can reduce possible local effects. Five minutes after treatment, cheek pouches of each animal were rinsed using tap water, in order to minimize the contact of the test