THINK FLT3 ONE MORE TIME

AML: **DEVASTATING**

WITH A FLT3 MUTATION: **DISASTROUS**

IN PATIENTS WITH AML,
A *FLT3-ITD* mutation drives progression and may lead to lower patient survival.[1-3]

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Prescribing information for: XOSPATA™ 40 mg film coated tablets (gilteritinib). **Indications:** Gilteritinib is indicated as monotherapy for the treatment of adult patients who have relapsed or refractory acute myeloid leukaemia (AML) with a FLT3 mutation. **Posology and administration:** Treatment with gilteritinib should be initiated and supervised by a physician experienced in the use of anti-cancer therapies. Before taking gilteritinib, relapsed or refractory AML patients must have confirmation of FMS-like tyrosine kinase 3 (FLT3) mutation (internal tandem duplication [ITD] or tyrosine kinase domain [TKD]) using a validated test. The recommended starting dose is 120 mg gilteritinib (three 40 mg tablets) orally once daily, with or without food, swallowed whole with water and should not be broken or crushed. Gilteritinib should be administered at about the same time each day. See *Special warnings and precautions for use* section on tests to be conducted prior to initiation e.g. blood chemistries, ECG & pregnancy test. Treatment should continue until the patient is no longer clinically benefiting from gilteritinib or until unacceptable toxicity occurs. Response may be delayed; therefore, continuation of treatment at the prescribed dose for up to 6 months should be considered to allow time for a clinical response. In the absence of a response (patient did not achieve a composite complete remission (CRc) after 4 weeks of treatment), the dose can be increased to 200 mg (five 40 mg tablets) once daily, if tolerated or clinically warranted. Gilteritinib may be re-initiated in patients following haematopoietic stem cell transplantation (HSCT). Treatment can be resumed 30 days after HSCT if engraftment was successful, the patient did not have grade ≥2 acute graft versus host disease and was in CRc. Elderly: No dose adjustment is required in patients ≥65 years of age. Gilteritinib is not recommended for use in patients with severe (Child-Pugh Class C) hepatic impairment. Please refer to SPC, section 4.2 for full instructions for use in hepatic & renal impairment. Paediatric population: The safety and efficacy of gilteritinib in children aged below 18 years has not yet been established. No data are available. Due to in vitro binding to 5HT₂ᵦ, there is a potential impact on cardiac development in patients less than 6 months of age. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients listed in section 6.1 of the SPC. **Special warnings and precautions for use:** Differentiation syndrome: Gilteritinib has been associated with differentiation syndrome. Differentiation syndrome is associated with rapid proliferation and differentiation of myeloid cells and may be life-threatening or fatal if not treated. Symptoms and clinical findings of differentiation syndrome include fever, dyspnoea, pleural effusion, pericardial effusion, pulmonary oedema, hypotension, rapid weight gain, peripheral oedema, rash, and renal dysfunction. If differentiation syndrome is suspected, corticosteroid therapy should be initiated along with haemodynamic monitoring until symptom resolution. If severe signs and/or symptoms persist for more than 48 hours after initiation of corticosteroids, gilteritinib should be interrupted until signs and symptoms are no longer severe. Corticosteroids can be tapered after resolution of symptoms and should be administered for a minimum of 3 days. Symptoms of differentiation syndrome may recur with premature discontinuation of corticosteroid treatment. Resume gilteritinib at the same dose when signs and symptoms improve to Grade 2 or lower. Posterior reversible encephalopathy syndrome: There have been reports of posterior reversible encephalopathy syndrome (PRES) in patients receiving gilteritinib. PRES is a rare, reversible, neurological disorder which can present with rapidly evolving symptoms including seizure, headache, confusion, visual and neurological disturbances, with or without associated hypertension and altered mental status. If PRES is suspected, it should be confirmed by brain imaging, preferably magnetic resonance imaging (MRI). Discontinuation of gilteritinib in patients who develop PRES is recommended. Prolonged QT interval: Gilteritinib has been associated with prolonged cardiac ventricular repolarisation (QT interval). QT prolongation can be observed in the first three months of treatment with gilteritinib. Therefore, ECG should be performed prior to initiation of treatment, on day 8 and 15 of cycle 1, and prior to the start of the next three subsequent months of treatment. Caution is warranted in patients with relevant cardiac history. Hypokalaemia or hypomagnesaemia may increase the QT prolongation risk. Hypokalaemia or hypomagnesaemia should therefore be corrected prior to and during gilteritinib treatment. Gilteritinib should be interrupted in patients who have a QTcF >500 msec. The decision to re-introduce gilteritinib treatment after an event of QT prolongation should be based on careful consideration of benefits and risks. Resume gilteritinib at a reduced dose (from 120 mg to 80 mg or from 200 mg to 120 mg) when QTcF interval returns to within 30 msec of baseline or ≤480 msec. Patients with QTcF interval increase by >30 msec on day 8 of cycle 1 should have a further ECG on day 9; if QTcF increase is confirmed gilteritinib dose should be reduced to 80 mg. If gilteritinib is re-introduced at a reduced dose, ECG should be performed after 15 days of dosing, and prior to the start of the next three subsequent months of treatment. In clinical studies, 12 patients had QTcF >500 msec. Three patients interrupted and re-initiated treatment without recurrence of QT prolongation. Pancreatitis: There have been reports of pancreatitis. Patients who develop signs and symptoms suggestive of pancreatitis should be evaluated and monitored. Gilteritinib should be interrupted and can be resumed at a reduced dose (reduced from 120 mg to 80 mg or from 200 mg to 120 mg) when the signs and symptoms of pancreatitis have resolved. Toxicity: If the patient experiences other Grade 3 or higher toxicity considered related to treatment, interrupt

treatment until the toxicity resolves or improves to Grade 1. If deemed clinically appropriate gilteritinib can be resumed at a reduced dose (reduced from 120 mg to 80 mg or from 200 mg to 120 mg). Interactions: Co-administration of CYP3A/P-gp inducers may lead to decreased gilteritinib exposure and consequently a risk for lack of efficacy. Therefore, concomitant use of gilteritinib with strong CYP3A4/P-gp inducers should be avoided. Caution is required when concomitantly prescribing gilteritinib with medicinal products that are strong inhibitors of CYP3A, P-gp and/or breast cancer resistant protein (BCRP) (such as, but not limited to, voriconazole, itraconazole, posaconazole and clarithromycin) because they can increase gilteritinib exposure. Alternative medicinal products that do not strongly inhibit CYP3A, P-gp and/or BCRP activity should be considered. In situations where satisfactory therapeutic alternatives do not exist, patients should be closely monitored for toxicities during administration of gilteritinib. Gilteritinib may reduce the effects of medicinal products that target 5HT₂ᵦ receptor or sigma nonspecific receptors. Therefore, concomitant use of gilteritinib with these products should be avoided unless use is considered essential for the care of the patient. Embryofoetal toxicity and contraception: Pregnant women should be informed of the potential risk to a foetus. Females of reproductive potential should be advised to have a pregnancy test within seven days prior to starting treatment with gilteritinib and to use effective contraception during treatment with gilteritinib and for at least 6 months after stopping treatment. Women using hormonal contraceptives should add a barrier method of contraception. Males with female partners of reproductive potential should be advised to use effective contraception during treatment and for at least 4 months after the last dose of gilteritinib. **Interactions:** Gilteritinib is primarily metabolised by CYP3A enzymes, which can be induced or inhibited by a number of concomitant medicinal products. See Special Warnings and Precautions for Use section above for further information on this and the effects of gilteritinib on products that target 5HT₂ᵦ receptor or sigma nonspecific receptors. Gilteritinib as an inhibitor or inducer: gilteritinib is not an inhibitor or inducer of CYP3A4 or an inhibitor of MATE1 in vivo. Gilteritinib is an inhibitor of P-gp, BCRP and OCT1 (organic cation transporter 1) in vitro. As no clinical data are available, it cannot be excluded that gilteritinib could inhibit these transporters at a therapeutic dose. Caution is advised during co-administration of gilteritinib with substrates of P-gp (e.g., digoxin, dabigatran etexilate), BCRP (e.g., mitoxantrone, methotrexate, rosuvastatin) and OCT1 (e.g., metformin). **Fertility, pregnancy and lactation:** Pregnancy: Gilteritinib is not recommended during pregnancy and in women of childbearing potential not using effective contraception. See Special Warnings and Precautions for Use section above for information on pregnancy testing and contraception. Breastfeeding: Breastfeeding should be discontinued during treatment with gilteritinib and for at least two months after the last dose. Fertility: There are no data on the effect of gilteritinib on human fertility. **List of adverse reactions:** Prescribers should consult the SPC for full information on adverse events **List of adverse reactions:** Very common (≥1/10): Dizziness, Hypotension, Cough, Dyspnoea, Diarrhoea, Nausea, Constipation, Alanine aminotransferase increased, Aspartate aminotransferase increased, Blood creatine phosphokinase increased, Blood alkaline phosphatase increased, Pain in extremity, Arthralgia, Myalgia, Fatigue, Peripheral oedema and Asthenia. Common (≥1/100 to <1/10): Anaphylactic reaction, Electrocardiogram QT prolonged, Pericardial effusion, Pericarditis, Cardiac failure, Differentiation syndrome, Musculoskeletal pain, Acute kidney injury and Malaise. Serious adverse reactions: The most frequent serious adverse reactions noted from evaluation of 319 patients with relapsed or refractory AML who have received at least one dose of 120 mg gilteritinib were acute kidney injury, diarrhoea, ALT increased, dyspnea, AST increased and hypotension. Other clinically significant serious adverse reactions included differentiation syndrome, electrocardiogram QT prolonged and posterior reversible encephalopathy syndrome. **Overdose:** There is no known specific antidote for gilteritinib. In the event of an overdose, treatment should be stopped. Patients must be closely monitored for signs or symptoms of adverse reactions, and appropriate symptomatic and supportive treatment initiated, taking into consideration the long half-life estimated at 113 hours. **Cost (excluding VAT):** United Kingdom (UK): XOSPATA 40 mg film-coated tablets x 84: £14,188.00. **Legal classification:** POM. **Marketing authorisation number:** Great Britain (GB): PLGB 00166/0425. Northern Ireland NI: EU/1/19/1399/001. **Marketing authorisation holder:** GB: Astellas Pharma Ltd., 300 Dashwood Lang Road, Bourne Business Park, Addlestone, United Kingdom, KT15 2NX. NI: Astellas Pharma Europe B.V. Sylviusweg 62, 2333 BE Leiden, The Netherlands. **Date of preparation:** March 2023. **Document number:** MAT_UK_XOS_2023_00039. **Further information available from:** Astellas Pharma Ltd., Medical Information: 0800 783 5018.

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store. Adverse events should also be reported to Astellas Pharma Ltd. on 0800 783 5018.

AML=acute myeloid leukemia; FLT3=FMS-like tyrosine kinase 3; ITD=internal tandem duplication.

References: 1. Chevallier P, et al. Leukemia 2011;25(6):939-44. **2.** Gale RE, et al. Blood 2008;111(5):2776-84. **3.** Smith CC, et al. Nature 2012;485(7397):260-3.

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MAT-UK-XOS-2023-00023 | March 2023

SHORT REPORT

Subpopulations of CD34-positive haemopoietic progenitors in fetal blood

BASKARAN THILAGANATHAN, KYPROS H. NICOLAIDES AND GARETH MORGAN*
*The Harris Birthright Research Centre for Fetal Medicine, King's College Hospital School of Medicine, London, and *Department of Immunology, Institute of Child Health, London*

Summary. Flow cytometry was used to determine the percentage and number of circulating CD34$^+$ cells in fetal blood from 100 pregnancies at 13–38 weeks gestation. When expressed as a percentage of the total number of lymphocytes, the proportion of CD34$^+$ cells decreased exponentially from a mean of 11·1% (9·2 × 10^7/l) at 13 weeks to 1·0% (3·0 × 10^7/l) at 38 weeks ($r = 0.751$,

$P < 0.0001$). The use of primitive fetal blood CD34$^+$ progenitor cells for prenatal somatic gene therapy may have distinct advantages over postnatal somatic gene therapy.

Keywords: fetal blood, CD34$^+$ cells.

Stem cell transplantation using cells isolated either from adult marrow or peripheral blood is now the established method of treatment of several hereditary disorders of haemopoiesis or metabolism, and malignancies of haemopoietic origin. The CD34 antigen has been used to identify a subpopulation of bone marrow and peripheral blood cells that are enriched for colony-forming activity *in vitro*, and are capable of complete haemopoietic reconstitution after engraftment into lethally-irradiated animals (Berenson *et al*, 1988). In postnatal life, CD34$^+$ cells are present in 1·8% of bone marrow and 0·2% of peripheral blood (Bender *et al*, 1991). In addition to CD34, the co-expression of cell-surface antigens such as HLA-DR, CD33 and other lineage-specific antigens are said to vary with maturation, loss of pluripotent and self-renewing capacity and altered sensitivity to growth factors (Caux *et al*, 1989; Andrews *et al*, 1989; Pierelli *et al*, 1993). Previous postnatal studies of these CD34$^+$ cells have been hampered by their low frequency and the need for *in vitro* culture techniques. The purpose of this study was to quantify the number of CD34$^+$ cells and subpopulations in fetal blood with advancing gestation in order to investigate haematological differentiation and development of fetal stem cells.

Correspondence: Dr B. Thilaganathan, Department of Obstetrics and Gynaecology, St Bartholomew's Homerton, Homerton Row, London E9 6SR.

PATIENTS AND METHODS
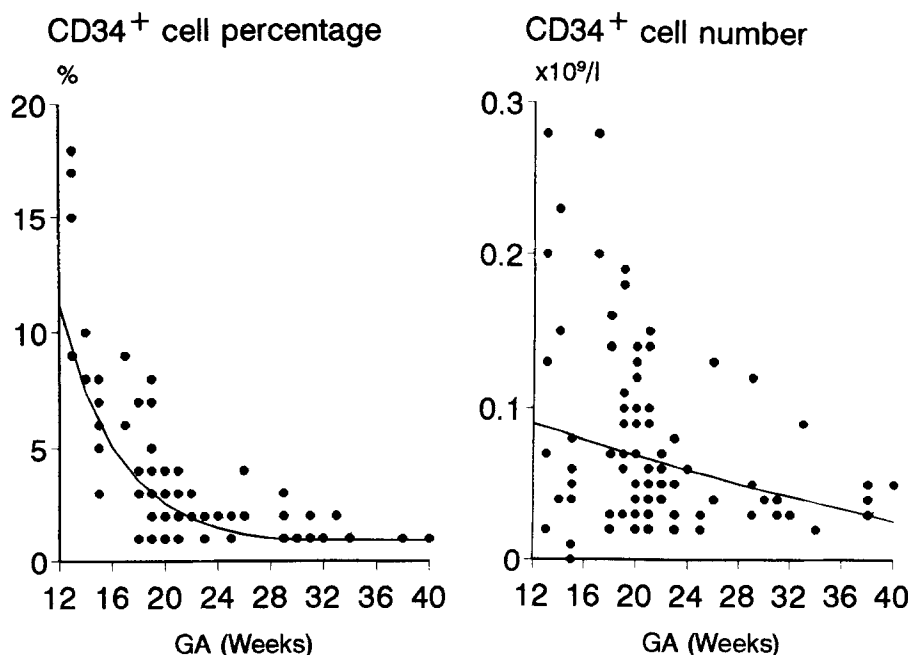
In a cross-sectional study of 100 pregnancies, fetal blood samples were obtained either by cordocentesis at 17–38 weeks gestation ($n = 82$), or by fetal cardiocentesis, from women undergoing elective termination of pregnancy for social indications at 13–17 weeks gestation ($n = 18$). In all cases the fetal karyotype was normal and the fetal haemoglobin concentration and white cell count were within the appropriate reference range for gestation.

In each case, gestation was determined from the maternal menstrual history and confirmed by an ultrasound scan in early pregnancy. Fetal blood samples (180 μl) were collected into 20 μl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l sodium chloride) and the full blood count was determined using a Coulter S-Plus counter (Coulter Electronics, Luton, U.K.). Blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Blood samples (0·5 ml) were also collected into heparinized syringes for enumeration of fetal lymphocyte subsets, which was performed on the day of sampling.

Fluorescein–isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal anti-human antibodies (Becton Dickinson UK Ltd, Oxford, U.K.) were used for simultaneous two-colour determination of lymphocyte subpopulations using CD34-FITC, HLA-DR, CD33 and α-glycophorin-PE. The whole-blood method was used for staining the cells with monoclonal antibody. Cytometric analysis was carried out using a FACScan and Consort 32 software (Becton

CD34$^+$ cell percentage

CD34$^+$ cell number

Fig 1. Relation between fetal CD34$^+$ cell percentage and number versus the length of gestation (CD34$^+$ percentage: $r = 0.751$, $P < 0.0001$; CD34$^+$ number: $r = -0.291$, $P < 0.01$).

Dickinson, Oxford, U.K.). Samples were gated using forward angle and 90° light-scattering properties to exclude granulocytes, monocytes and platelets. Gated cells were analysed with CD14/CD45 (monocyte/leucocyte marker) to confirm that they were lymphoid in origin. Control staining of fetal cells with anti-mouse monoclonal IgG$_{2a}$-PE/IgG$_1$-FITC was performed on each sample, and background readings of $< 1\%$ were obtained. A minimum of 5000 cells were acquired in the lymphocyte fraction and analysed to calculate the percentage of CD34$^+$ cells. The absolute number of CD34$^+$ cells was derived from the lymphocyte differential count on the blood film and the percentage of CD34$^+$ cells.

Regression analysis was used to determine the significance of any association between the number and percentage of CD34$^+$ cells and gestational age. Logarithmic transformation was used to make the data Gaussian. Mann-Whitney U tests were used to determine the significance of any differences between groups.

Table I. The percentage co-expression (± 1 SD) of HLA-DR, CD33 and α-glycophorin on CD34$^+$ fetal peripheral blood mononuclear cells obtained at 13–15 weeks gestation ($n = 12$).

Monoclonal antibody	Percentage co-expression with CD34
HLA-DR	70 ± 18
CD33	82 ± 23
α-Glycophorin	13 ± 12

RESULTS

The percentage and number of fetal CD34$^+$ cells decreased with gestational age (Fig 1: CD34$^+$ percentage: $r = 0.751$, $P < 0.0001$; CD34$^+$ number: $r = -0.291$, $P < 0.01$). The co-expression of HLA-DR, CD33 and α-glycophorin on CD34$^+$ cells before 16 weeks gestation is shown in Table I. The expression of HLA-DR on CD34$^+$ cells at 13–17 weeks (median 83%, range 44–100%, $n = 18$) was significantly lower ($z = 5.28$, $P < 0.0001$) than at 18–38 weeks gestation (median 100%, range 50–100%, $n = 82$).

DISCUSSION

This study demonstrated that both the total number and percentage of CD34$^+$ cells in the fetal circulation decrease exponentially with advancing gestation. The number of CD34$^+$ cells detected at 36–38 weeks in this study is similar to data from studies of CD34$^+$ cells in cord blood after delivery (Reisbach *et al*, 1993). The finding of high levels of CD34$^+$ haemopoietic progenitor cells in early pregnancy (12–20 weeks), when bone marrow haemopoiesis is being established, supports the hypothesis that developing bone marrow is colonized by circulating stem cells (Moore & Metcalf 1970; Barnes *et al*, 1964).

The finding that there are more CD34$^+$ DR$^-$ cells in early than in late pregnancy is in keeping with postnatal data from stem cell cultures which have demonstrated that the acquisition of the HLA-DR antigen is associated with the loss of multilineage capacity and the maintenance of proliferative capacity of the stem cells (Caux *et al*, 1989). This is supported by the finding that most of the CD34$^+$ cells are

also CD33^{+}, a phenotype associated with the progressive loss of self-renewal capacity in adult long-term marrow cultures (Andrews *et al*, 1989). Although the level of CD33 co-expression on CD34^{+} cells is equivalent to that found in adult peripheral blood, HLA-DR expression is lower in early pregnancy and reaches adult levels at term (Bender *et al*, 1991; Pierelli *et al*, 1993). Previous studies have demonstrated that fetal cells are more responsive to stimulation by erythropoietin and colony-stimulating factors than adult cells under similar conditions (Linch *et al*, 1982; Kidoguchi *et al*, 1978). Furthermore, erythropoietin stimulation of fetal cells results in the production of predominantly HbF, whereas adult cells produce HbA (Kidoguchi *et al*, 1978). These findings suggest that fetal CD34^{+} progenitors are more primitive than adult cells and retain a multilineage capacity despite some phenotypic similarity.

In future, fetal CD34^{+} cells may be used for prenatal somatic gene therapy. Many candidate diseases can now be diagnosed by chorion villus biopsy at 11–12 weeks gestation. Fetal blood sampling could then be undertaken by cordocentesis for the isolation of CD34^{+} cells and for the reintroduction of these cells after transfection. The advantage of this form of fetal therapy are early treatment in a sterile environment before the onset of disease-related complications, intrauterine monitoring of the effects of gene therapy, and repeated infusions of transfected cells if necessary. Furthermore, the use of primitive fetal CD34^{+} progenitor cells which are colonizing the bone marrow and have a high self-renewal capacity may result in life-long expression of the transfected gene.

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