FLT3 ligand in AML: a simple test with deep implications

Running title: FLT3 Ligand in AML

Keywords: FLT3 Ligand, Acute Myeloid Leukemia

Authors: Pierre Peterlin[1,2], Patrice Chevallier[1,2], Steven Knapper[3] and Matthew Collin[4]

1-Hematology Clinic, CHU Nantes, France

2-CRCINA, INSERM, CNRS, Université d'Angers, Université de Nantes, Nantes, France.

3-Division of Cancer & Genetics, School of Medicine, Cardiff University, Cardiff, UK.

4-Newcastle University Translational and Clinical Research Institute and NIHR Newcastle Biomedical Research Centre, Newcastle University, Newcastle Upon Tyne NE2 4HH, UK

All authors have no conflicts of interest to disclose.

Contact info:

Dr. Pierre Peterlin (corresponding author)

Mail to: pierre.peterlin@chu-nantes.fr

Dr. Matthew Collin (corresponding author)

Mail to: matthew.collin@newcastle.ac.uk

Dr Patrice Chevallier, Patrice.chevallier@chu-nantes.fr

Dr. Steven Knapper, KnapperS@cardiff.ac.uk

Abstract

In contrast to Fms-like tyrosine kinase 3 (FLT3), the influence of FLT3 ligand (FLT3L) on acute myeloid leukemia (AML) biology and disease prognosis has been poorly described. Here we provide an overview of the role played by FLT3L in AML. While being a cytokine implicated in the regulation of hematopoiesis, both in normal situation and after intensive chemotherapy, FLT3L has also a role in enhancing proliferation, inhibiting apoptosis and conferring resistance to FLT3 inhibitors in AML. Moreover, recent independent data show how its measurement may be helpful in the disease management. Indeed, FLT3L could provide a low cost, rapid and non-invasive assessment of chemosensitivity and blast clearance that has robust prognostic significance for patients with AML.

Introduction

Cytokines are a heterogeneous group of proteins that are produced in response to an activation signal, acting through interaction with specific receptors that conduct paracrine, endocrine, juxtacrine or intracrine effects at the cellular level. They are implicated in key processes including cell growth and proliferation, pro-inflammatory and anti-inflammatory responses and adaptive immunity. Of particular interest for hematologists are cytokines that could be involved in leukemogenesis, especially when considering such an heterogeneous disease as acute myeloid leukemia (AML). In this review, we will focus on the Fms-like tyrosine kinase 3 ligand (FLT3L). Since its cloning in the early 90s,[1] it has become increasingly apparent that FLT3L is an important regulator of hematopoiesis. It is expressed or produced, as a transmembrane or soluble protein, by a variety of cells including hematopoietic and marrow stromal cells[2] In fact, FLT3L mRNA is widely expressed but FLT3L protein expression is restricted to T cells and bone marrow and thymic fibroblasts.[3] As a key regulator of hematopoiesis, the main factor inducing FLT3L expression is bone marrow aplasia induced after cytotoxic chemotherapy and or radiotherapy.[4,5]

In combination with other growth factors, FLT3L stimulates proliferation and development of stem cells, myeloid and lymphoid progenitor cells, dendritic cells (DC) and natural killer cells.[6] Its receptor, FLT3 (also known as CD135, fetal liver kinase 2 (FLK2) and stem cell tyrosine kinase 1 (STK1), expressed almost exclusively in the hematopoietic compartment, is a tyrosine kinase receptor considered as an important growth and differentiation factor for several hematopoietic lineages (myeloid, lymphoid and DC).[7] The binding of FLT3L to FLT3 induces the dimerization and activation of its intrinsic tyrosine kinase domain. This leads to the auto-phosphorylation of FLT3 on several tyrosine residues which constitute high affinity binding sites for transduction signal molecules.[8]

FLT3 is frequently mutated or overexpressed in AML. Two types of FLT3-activating mutations have been described; internal tandem duplications of the juxta-membrane domain (FLT3-ITD) and point mutations (especially D835 mutation) in the tyrosine kinase domain (FLT3-TKD) are found in about one-third of AML cases.[9] FLT3 mutations, especially ITDs, are associated with a poor prognosis.[10] High FLT3 expression, independent of the presence or absence of FLT3 mutations, is also commonly found in AML and seems to be correlated with FAB classification (strong correlation with M5 sub-group).[11] In contrast to FLT3, the influence of its ligand FLT3L on AML biology (role in leukemogenesis and chemo-resistance) and disease prognosis has been less well-described. Following observations reported recently by our two teams in France and the UK, the aim of this mini review is to provide an overview of the role played by FLT3L in AML and how its measurement may be practically helpful in the disease management.

FLT3L in AML

A role in enhancing proliferation and inhibiting apoptosis

Outside its normal production, FLT3L may be expressed by leukemic cells and lead to their proliferation[12] through autocrine or paracrine processes, [13–18] FLT3L caused a proliferative response in a significant percentage of AML cases (22-90%; mean 53%).[12] These findings are irrespective of

whether patients have FLT3 mutations or not.[19] The role of FLT3L on leukemic proliferation has been especially suggested in M4, M5 and M6 FAB AML which appear more sensitive to its action, explaining in part the hyperleucocytosis observed in these sub-types of AML[20,21] FLT3L has been also shown to be play an anti-apoptotic role, especially through the downregulation of Bax protein in leukemic cells.[19,22–24] Furthermore, aberrant FLT3L expression may predispose hematopoietic precursor cells to leukemic transformation.[18] However, data suggest that these effects of FLT3L on the growth of malignant hematopoietic cells may be dependent on the action of other cytokines;[25,26] for example, synergistic activities have been observed when FLT3L is combined with G-CSF, GM-CSF, IL-3, or stem cell factor (SCF) on the growth of myeloid leukemia cells.[26]

A role in conferring resistance to FLT3 inhibitors

It is now well-recognized that mutated FLT3 represents a therapeutic target in AML, and testing for the presence of ITD or TKD mutations at diagnosis or relapse forms a central part of the recommended management of such patients.[27–29] Several first and second generation FLT3 inhibitors are currently in clinical use, either alone or in combination with chemotherapy, and are currently applied depending on the disease stage and regional regulatory approvals. A number of mechanisms of acquired resistance to FLT3 inhibitors have now been proposed, with overexpression of FLT3L being one suggested [30,31] Of note, this phenomenon does not appear to be impacted by the co-expression of both wild-type and mutated FLT3 within leukemia cells.[32] The mechanism by which overexpression of FLT3L induces resistance to FLT3 inhibitors remains to be fully clarified, although some studies report an activation of AKT and/or ERK signaling pathways via a stimulation of wild type FLT3, leading to a protective effect against FLT3 inhibitors.[31,32] Interestingly, dual inhibition of AKT/FLT3-ITD proteins by A674563, an AKT inhibitor, overcomes FLT3L-induced drug resistance in FLT3-ITD positive AML.[33] Another *in vitro* study has shown that FLT3L, in the absence of FLT3 inhibitors, leads to further activation of mutated FLT3, the authors speculating that FLT3L-targeted therapy may be an important adjuvant in the treatment of FLT3 mutated AML.[34] Taken together, even if the favorable

effect of FLT3L on proliferation of FLT3-ITD cells may depend on the heterozygosity of FLT3 ITD (at least in mice),[35] FLT3L-induced resistance to FLT3 inhibitors appears to be one of the main obstacles to effective patient treatment. These limitations may be overcome by more highly FLT3-directed drugs with more favorable pharmacokinetic properties. Data from a xenograft model suggest that gilteritinib, could overcome this resistance, through its ability to equally inhibit proliferation of both wild type and FLT3-ITD AML.[36]

A role in recuperation of normal hematopoiesis after intensive chemotherapy

Interest in FLT3L in leukemia dates back approximately twenty years. In 2001, Bruserud et al. were the first to evaluate the plasma level of FLT3L in 13 leukemia patients following intensive chemotherapy, showing that initial levels in leukemic patients were comparable to those of healthy controls, with levels then increasing during treatment.[37] However, this study included both AML and acute lymphoblastic leukemia (ALL) and FLT3L levels were only evaluated in patients receiving consolidation therapy (*i.e.* having already achieved disease remission with initial therapy).

Ten years later, Sato et al retrospectively showed in AML patients with FLT3 mutations i) that plasma FLT3L levels were significantly lower at diagnosis than at relapse, ii) that plasma FLT3L levels can be implicated in the mechanisms of resistance to FLT3 inhibitors, including lestaurtinib, quizartinib and sorefenib (as discussed above) and iii) that plasma FLT3L levels rise following chemotherapy with even greater increases after multiple rounds of chemotherapy. These observations were restricted to FLT3L concentrations measured on day 15 of each cycle of chemotherapy.[31] In one more extensively-studied newly-diagnosed case, it was shown that plasma FLT3L concentrations rose after the start of treatment, peaking at day 15 and then decreasing to baseline by day 25. Subsequently, others have also observed an increase of FLT3L concentrations during the period of aplasia following radiotherapy or chemotherapy for AML, FLT3L levels being inversely correlated with neutrophil and

platelet counts, rising before the neutrophil nadir then decreasing following recovery of normal blood-cell populations.[4,37–41]

A role as a prognostic factor in AML

In 2015, in Nantes we investigated the kinetics of FLT3L levels in a Phase 1 study testing a radio-immunotherapy regimen for relapsed/refractory ALL, demonstrating that only the clinical responders displayed sustained increased soluble FLT3L plasma concentrations.[42] In light of these data we went on to design a prospective single center study to expand these results in a larger cohort of leukemic patients (ALL and AML) at diagnosis, also studying the prognostic impact of plasma FLT3L concentrations on outcome in these patients (FLAM/FLAL study; University Hospital of Nantes, Nantes, France, www.ClinicalTrials.gov NCT02693899). No significant information emerged from ALL or post-induction AML samples, but three different plasma FLT3L kinetic profiles were revealed during induction in AML patients (n=62): i) sustained increase from days 1 to 22 ('FLI' group), ii) increase from days 1-15, followed by decrease at day 22 ('FLD' group), and iii) stagnation of low FLT3L levels ('FLL' group). Significantly more refractory and relapsed patients were found in the FLL group (n=14) compared to FLD (n=22) and FLI (n=26) groups (p=0.0007 and p=0.0009, respectively). In univariate analysis, 2-year progression-free (PFS) and overall (OS) survival were better for the FLI group (p<0.001 and p=0.09 respectively). In multivariate analysis, the FLT3L induction kinetic profile remained the most powerful factor associated with PFS (HR: 3.62; 95%CI: 1.65-7.94, p=0.001) and was the sole factor associated with OS (HR:2.60; 95% CI:1.12-6.07, p=0.02). We thus concluded that plasma FLT3L kinetics during induction provides a powerful new early prognostic parameter in AML that carries the potential to be used in daily practice to identify high-risk patients.[43]

Simultaneously in the UK, retrospective analysis of the UK National Cancer Research Institute AML17 trial coupled with the addition of single center data from the UK (Newcastle) and US

(Baltimore), established that, for 140 AML FLT3 mutated patients, achievement of CR following intensive induction therapy was associated with higher day 26 FLT3L level ($p<0.0001$). Day 26 FLT3L concentration was also associated with longer term outcomes: a FLT3L level of $\leq$291 pg/mL was associated with inferior event-free survival (EFS, $p=0.01$), and FLT3L<1185 pg/mL was associated with higher OS ($p=0.0119$). Patients with sustained clinical responses maintained increased FLT3L levels, whilst treatment refractory patients showed persistently undetectable FLT3L concentrations, concluding that measurement of FLT3L is a non-invasive test that has the potential to inform clinical decision making in AML patients.[44]

Hypothesis to explain the prognostic impact of FLT3L in AML

It remains fundamentally unclear why FLT3L concentrations increase in some patients but not in others; plasma/serum FL concentration is approximately 100pg/ml in healthy individuals and has been shown, as mentioned before, to correlate inversely with neutrophil and platelet counts during recovery from the bone marrow aplasia that follows radiotherapy or chemotherapy[4,37–41]. It has also been reported that extremely high levels of FLT3L are found in patients presenting with cytopenias due to GATA2 mutation.[45] These results suggest that the level of soluble FLT3L is inversely correlated with the frequency of progenitors expressing its receptor, FLT3 (CD135) (**Figure 1A**) and is therefore an indicator of progenitor cell mass (inversely related to the rate of consumption). In keeping with this, normal Flt3L was observed in some patients with acute promyelocytic leukemia, which typically has low FLT3/CD135 expression. Thus, one could postulate that the rise in FLT3L concentration seen during effective induction chemotherapy reflects the normal reaction of the hematopoietic tissue to the clearance of malignant blasts and the subsequent need to restore normal blood counts[2]. Conversely, the low levels of FLT3L seen in cases with persistence of leukemic blasts has several possible interpretations: i) continuous consumption of FLT3L by leukemic blasts expressing FLT3/CD135; ii) incapacity of the microenvironment to produce FLT3L for restoration of the normal hematopoiesis; iii) interactions between leukemic blasts and the microenvironment preventing normal production of

FLT3L. Further investigations are needed to better decipher the precise mechanisms underlying persistence of low FLT3L levels in high-risk AML patients.

Beside its critical role in normal hematopoiesis,[2] FLT3L may also stimulate antitumor immunity against AML.[46] DC cells appear to be of particular interest in this context. Indeed, it has been shown that leukemic DCs generated in the presence of FLT3L have the capacity to stimulate an autologous leukemia-specific cytotoxic T cell response in AML patients.[47] Also, tumor-pulsed or in vivo FLT3 ligand-generated DCs provide protection against AML in non-transplanted or syngeneic bone marrow-transplanted mice.[48,47] Finally, others have shown that DCs engineered to secrete FLT3L have the capacity to increase tumor-specific cytotoxic T lymphocytes (CTL) and non-specific NK responses in a mouse model.[49] All of these studies suggest a critical role of FLT3L in inducing anti-tumor immunity, especially in AML. The increase of FLT3L level observed in good-risk AML patients may explain that their better outcomes may occur consequent on the anti-leukemic activity triggered by FLT3L itself, also possibly suggesting that FLT3L itself may be clinically useful as a therapeutic adjunct in combination with chemotherapy.

A role in the management of AML patients

Both of our studies [43,44] arrived separately at the conclusion that the evaluation of FLT3L level can provide a low cost, rapid and non-invasive assessment of chemo-sensitivity and blast clearance during intensive induction. Strikingly, these results were obtained in patients with wild type FLT3 AML (French study) as well as FLT3 mutated AML (UK study), strengthening the robust prognostic significance of this parameter. In the era of molecular genetic testing and minimal residual disease evaluation, it is perhaps surprising that the simple ELISA measurement of a growth factor has such potential. However, as mentioned before, FLT3L is a key regulator of hematopoiesis and arguably one of the most relevant molecules in AML.[13]

There are several ways that FLT3L might become useful in the management of patients with AML. First, most patients presenting with AML have profoundly depressed levels of FLT3L. Conversely, patients with aplastic anemia or hypoplasia have very elevated serum FLT3L concentrations.[5,50,51] Thus, FLT3L may help discriminate between AML presenting with cytopenias and aplastic anemia or other secondary bone marrow failure syndromes. Another potential application is the monitoring of ambulatory patients with myelodysplastic syndromes (MDS), for whom it may be difficult to predict evolution to AML. FL levels are initially normal in MDS but would be expected to fall with increased blast numbers during progression to AML, as described in the evolution of patients with germline GATA2 mutations.[45] Thus, even though subsequent diagnostic confirmation would be necessary, a decreasing level of FLT3L, in the context of falling peripheral blood counts, may be a useful monitoring test to trigger more definitive assessment with bone marrow biopsy. Conversely, a normal or increasing level of FLT3L may avoid such an invasive procedure, especially in older patients.

Second, FLT3L may help also to stratify AML patients. During induction, FLT3L levels were strongly associated with response, prognosis and outcome in both of our studies.[43,52] In the French study, a sustained elevated FLT3L concentration was associated with a higher rate of complete remission (CR) and the best outcome, while persisting low levels (<1000 pg/mL) were predictive of poor response and lower survival.[43] The UK NCRI AML17 trial only recorded a single FLT3L measurement at day 26 but also demonstrated a strong association with attainment of CR and had sufficient power to partition high and low event-free and overall (OS) survival groups.[44] Finally, in the French study, adjusting for age and ELN 2010 stratification,[53] the FLT3L kinetic profile was the most powerful factor independently associated with progression-free survival (HR: 3.62; 95%CI: 1,65-7,94, p=0,001) and was the sole factor independently associated with OS (HR: 2.60; 95%CI: 1.12-6,07, p=0.02).[43] These findings are strongly concordant as shown in **Figure 1B**. The data suggest that FLT3L measurement could provide a 'real-time' monitoring of blast clearance, potentially facilitating the discussion of rapid access to salvage strategies in patients, especially for those with persistent pancytopenia and persistently suppressed FLT3L levels , while documentation of a normal/high level

of FLT3L may provide reassurance to allow more time to wait for hematological recovery. Also, FLT3L could be used to drive clinical trials or to evaluate novel agents in AML.

Third, the measurement of FLT3L may have the potential to be used during long term follow up, for example after allotransplant. As predicted, conditioning regimens and resulting aplasia are associated with significantly elevated FLT3L which declines with engraftment.[44,54] The pre-transplant level of FLT3L does not appear to correlate with GVHD or outcome, but rapidly declining FLT3L may predict relapse, provided that relatively high frequency monitoring is possible.

Many questions remain concerning the utility of FLT3L in relation to genetic risk groups and minimal residual disease (MRD) status. In the UK study, measurements were taken only from patients with FLT3 mutations, randomized to a FLT3 inhibitor or placebo (which did not appear to influence the level of FLT3L). FLT3L appears to function in a completely complementary manner to MRD, reflecting the bulk level of progenitors/blasts rather than low level disease. This is not necessarily disadvantageous though, since our data suggest that the clearance of blasts by chemotherapy may be an independent and highly useful predictor of outcome. The UK group showed that FLT3L retained a strong association with survival after adjusting for MRD status (not quite reaching statistical significance), as illustrated by an 'elite survivor' group with both undetectable MRD and high FLT3L who had 100% OS.[44]

Conclusion

FLT3L appears to be a potentially powerful new prognostic factor in AML that may provide an inexpensive, rapid and non-invasive method of assessing remission status. Larger prospective studies will be required to confirm recent results whilst testing the independence of this marker alongside other variables known to predict outcomes in AML, and also in the context of different treatment regimens. Further elucidation of the mechanisms underlying the clinical impact of FL levels will require further detailed research, including leukemic and progenitor cells themselves, cytokine

production and the bone marrow microenvironment. In the meantime, this is an additional mode of disease assessment that is likely to be much appreciated by AML patient focus groups: the provision of a rapid, non-invasive and almost universal test to answer the question 'how am I doing?' during the long cytopenic phase that follows induction chemotherapy. Thus, if the FLT3L response is ultimately found to reflect baseline performance, genetic risk group and MRD status, the measurement of FLT3L has the potential to become the 'sedimentation rate' of leukemia; a cheap and simple means of determining the clinical trajectory on a daily basis. We just have to start measuring it.

References

1 Lyman SD, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B *et al.* Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. *Cell* 1993; **75**: 1157–1167.

2 Tsapogas P, Mooney CJ, Brown G, Rolink A. The Cytokine Flt3-Ligand in Normal and Malignant Hematopoiesis. *Int J Mol Sci* 2017; **18**. doi:10.3390/ijms18061115.

3 McClanahan T, Culpepper J, Campbell D, Wagner J, Franz-Bacon K, Mattson J *et al.* Biochemical and genetic characterization of multiple splice variants of the Flt3 ligand. *Blood* 1996; **88**: 3371–3382.

4 Bertho JM, Demarquay C, Frick J, Joubert C, Arenales S, Jacquet N *et al.* Level of Flt3-ligand in plasma: a possible new bio-indicator for radiation-induced aplasia. *Int J Radiat Biol* 2001; **77**: 703–712.

5 Wodnar-Filipowicz A, Lyman SD, Gratwohl A, Tichelli A, Speck B, Nissen C. Flt3 ligand level reflects hematopoietic progenitor cell function in aplastic anemia and chemotherapy-induced bone marrow aplasia. *Blood* 1996; **88**: 4493–4499.

6 Drexler HG, Quentmeier H. Mini ReviewFLT3: Receptor and Ligand. *Growth Factors* 2004; **22**: 71–73.

7 Matthews W, Jordan CT, Wiegand GW, Pardoll D, Lemischka IR. A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. *Cell* 1991; **65**: 1143–1152.

8 Kazi JU, Rönnstrand L. The role of SRC family kinases in FLT3 signaling. *The International Journal of Biochemistry & Cell Biology* 2019; **107**: 32–37.

9 Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood* 2002; **100**: 1532–1542.

10 Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; **129**: 424–447.

11 Kuchenbauer F, Kern W, Schoch C, Kohlmann A, Hiddemann W, Haferlach T *et al.* Detailed analysis of FLT3 expression levels in acute myeloid leukemia. *Haematologica* 2005; **90**: 1617–1625.

12 Dehmel U, Zaborski M, Meierhoff G, Rosnet O, Birnbaum D, Ludwig WD *et al.* Effects of FLT3 ligand on human leukemia cells. I. Proliferative response of myeloid leukemia cells. *Leukemia* 1996; **10**: 261–270.

13 Meierhoff G, Dehmel U, Gruss HJ, Rosnet O, Birnbaum D, Quentmeier H *et al.* Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia* 1995; **9**: 1368–1372.

14 Brasel K, Escobar S, Anderberg R, de Vries P, Gruss HJ, Lyman SD. Expression of the flt3 receptor and its ligand on hematopoietic cells. *Leukemia* 1995; **9**: 1212–1218.

15 Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia* 1996; **10**: 588–599.

16 Zheng R, Levis M, Piloto O, Brown P, Baldwin BR, Gorin NC *et al.* FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. *Blood* 2004; **103**: 267–274.

17 Weisel KC, Yildirim S, Schweikle E, Kanz L, Möhle R. Regulation of FLT3 and its ligand in normal hematopoietic progenitor cells. *Ann Hematol* 2009; **88**: 203–211.

18 Hawley TS, Fong AZ, Griesser H, Lyman SD, Hawley RG. Leukemic predisposition of mice transplanted with gene-modified hematopoietic precursors expressing flt3 ligand. *Blood* 1998; **92**: 2003–2011.

19 Bruserud Ø, Hovland R, Wergeland L, Huang T-S, Gjertsen BT. Flt3-mediated signaling in human acute myelogenous leukemia (AML) blasts: a functional characterization of Flt3-ligand effects in AML cell populations with and without genetic Flt3 abnormalities. *Haematologica* 2003; **88**: 416–428.

20 McKenna HJ, Smith FO, Brasel K, Hirschstein D, Bernstein ID, Williams DE *et al.* Effects of flt3 ligand on acute myeloid and lymphocytic leukemic blast cells from children. *Exp Hematol* 1996; **24**: 378–385.

21 Murohashi I, Yoshida K, Kishimoto K, Takahashi T, Wakao D, Jinnai I *et al.* Differential response to stem cell factor and Flt3 ligand by the FAB subtype in acute myeloid leukemia clonogenic cells. *J Interferon Cytokine Res* 2002; **22**: 335–341.

22 Lisovsky M, Estrov Z, Zhang X, Consoli U, Sanchez-Williams G, Snell V *et al.* Flt3 ligand stimulates proliferation and inhibits apoptosis of acute myeloid leukemia cells: regulation of Bcl-2 and Bax. *Blood* 1996; **88**: 3987–3997.

23 Meyer C, Drexler HG. FLT3 ligand inhibits apoptosis and promotes survival of myeloid leukemia cell lines. *Leuk Lymphoma* 1999; **32**: 577–581.

24 Demmerath E-M, Bohler S, Kunze M, Erlacher M. In vitro and in vivo evaluation of possible pro-survival activities of PGE2, EGF, TPO and FLT3L on human hematopoiesis. *Haematologica* 2019; **104**: 669–677.

25 Dehmel U, Quentmeier H, Drexler HG. Effects of FLT3 ligand on human leukemia cells. II. Agonistic and antagonistic effects of other cytokines. *Leukemia* 1996; **10**: 271–278.

26 Piacibello W, Fubini L, Sanavio F, Brizzi MF, Severino A, Garetto L *et al.* Effects of human FLT3 ligand on myeloid leukemia cell growth: heterogeneity in response and synergy with other hematopoietic growth factors. *Blood* 1995; **86**: 4105–4114.

27 Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; **129**: 424–447.

28 https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf. .

29 Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia* 2019; **33**: 299–312.

30 Yang X, Sexauer A, Levis M. Bone marrow stroma-mediated resistance to FLT3 inhibitors in FLT3-ITD AML is mediated by persistent activation of extracellular regulated kinase. *Br J Haematol* 2014; **164**: 61–72.

31 Sato T, Yang X, Knapper S, White P, Smith BD, Galkin S *et al.* FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. *Blood* 2011; **117**: 3286–3293.

32 Chen F, Ishikawa Y, Akashi A, Naoe T, Kiyoi H. Co-expression of wild-type FLT3 attenuates the inhibitory effect of FLT3 inhibitor on FLT3 mutated leukemia cells. *Oncotarget* 2016; **7**: 47018–47032.

33 Wang A, Wu H, Chen C, Hu C, Qi Z, Wang W *et al.* Dual inhibition of AKT/FLT3-ITD by A674563 overcomes FLT3 ligand-induced drug resistance in FLT3-ITD positive AML. *Oncotarget* 2016; **7**: 29131–29142.

34 Zheng R, Bailey E, Nguyen B, Yang X, Piloto O, Levis M *et al.* Further activation of FLT3 mutants by FLT3 ligand. *Oncogene* 2011; **30**: 4004–4014.

35 Kharazi S, Mead AJ, Mansour A, Hultquist A, Böiers C, Luc S *et al.* Impact of gene dosage, loss of wild-type allele, and FLT3 ligand on Flt3-ITD-induced myeloproliferation. *Blood* 2011; **118**: 3613–3621.

36 Kawase T, Nakazawa T, Eguchi T, Tsuzuki H, Ueno Y, Amano Y *et al.* Effect of Fms-like tyrosine kinase 3 (FLT3) ligand (FL) on antitumor activity of gilteritinib, a FLT3 inhibitor, in mice xenografted with FL-overexpressing cells. *Oncotarget* 2019; **10**: 6111–6123.

37 Bruserud O, Foss B, Petersen H. Hematopoietic growth factors in patients receiving intensive chemotherapy for malignant disorders: studies of granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and Flt-3 ligand (Flt3L). *Eur Cytokine Netw* 2001; **12**: 231–238.

38 Prat M, Frick J, Laporte J-P, Thierry D, Gorin N-C, Bertho J-M. Kinetics of plasma FLT3 ligand concentration in hematopoietic stem cell transplanted patients. *Leuk Lymphoma* 2006; **47**: 77–80.

39 Haidar JH, Bazarbachi A, Mahfouz R, Haidar HA, Jaafar H, Daher R. Serum Flt3 ligand variation as a predictive indicator of hematopoietic stem cell mobilization. *J Hematother Stem Cell Res* 2002; **11**: 533–538.

40 Sato T, Yang X, Knapper S, White P, Smith BD, Galkin S *et al.* FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. *Blood* 2011; **117**: 3286–3293.

41 Şahin M, Haznedaroğlu İC, Özbalcı D. Peripheral FLT-3 ligand levels as a pathobiological parameter duringthe clinical course of acute myeloid leukemia. *Turk J Med Sci* 2016; **46**: 1889–1893.

42 Chevallier P, Eugene T, Robillard N, Isnard F, Nicolini F, Escoffre-Barbe M *et al.* (90)Y-labelled anti-CD22 epratuzumab tetraxetan in adults with refractory or relapsed CD22-positive B-cell acute lymphoblastic leukaemia: a phase 1 dose-escalation study. *Lancet Haematol* 2015; **2**: e108-117.

43 Peterlin P, Gaschet J, Guillaume T, Garnier A, Eveillard M, Le Bourgeois A *et al.* FLT3 ligand plasma levels in acute myeloid leukemia. *Haematologica* 2019; **104**: e240–e243.

44 Milne P, Wilhelm-Benartzi C, Grunwald MR, Bigley V, Dillon R, Freeman SD *et al.* Serum Flt3 ligand is a biomarker of progenitor cell mass and prognosis in acute myeloid leukemia. *Blood Adv* 2019; **3**: 3052–3061.

45 Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N *et al.* The evolution of cellular deficiency in GATA2 mutation. *Blood* 2014; **123**: 863–874.

46 Liu Y, Huang H, Chen Z, Zong L, Xiang J. Dendritic cells engineered to express the Flt3 ligand stimulate type I immune response, and induce enhanced cytoxic T and natural killer cell cytotoxicities and antitumor immunity. *J Gene Med* 2003; **5**: 668–680.

47 Woiciechowsky A, Regn S, Kolb HJ, Roskrow M. Leukemic dendritic cells generated in the presence of FLT3 ligand have the capacity to stimulate an autologous leukemia-specific cytotoxic T cell response from patients with acute myeloid leukemia. *Leukemia* 2001; **15**: 246–255.

48 Pawlowska AB, Hashino S, McKenna H, Weigel BJ, Taylor PA, Blazar BR. In vitro tumor-pulsed or in vivo Flt3 ligand-generated dendritic cells provide protection against acute myelogenous leukemia in nontransplanted or syngeneic bone marrow-transplanted mice. *Blood* 2001; **97**: 1474–1482.

49 Liu Y, Huang H, Chen Z, Zong L, Xiang J. Dendritic cells engineered to express the Flt3 ligand stimulate type I immune response, and induce enhanced cytoxic T and natural killer cell cytotoxicities and antitumor immunity. *The Journal of Gene Medicine* 2003; **5**: 668–680.

50 Lyman SD, Seaberg M, Hanna R, Zappone J, Brasel K, Abkowitz JL *et al.* Plasma/serum levels of flt3 ligand are low in normal individuals and highly elevated in patients with Fanconi anemia and acquired aplastic anemia. *Blood* 1995; **86**: 4091–4096.

51 Molyneux G, Gibson FM, Whayman M, Turton JA. Serum FLT-3 ligand in a busulphan-induced model of chronic bone marrow hypoplasia in the female CD-1 mouse. *Int J Exp Pathol* 2008; **89**: 159–170.

52 Milne P, Wilhelm-Benartzi C, Grunwald MR, Bigley V, Dillon R, Freeman SD *et al.* Serum Flt3 ligand is a biomarker of progenitor cell mass and prognosis in acute myeloid leukemia. *Blood Adv* 2019; **3**: 3052–3061.

53 Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK *et al.* Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* 2010; **115**: 453–474.

54 Peterlin P, Gaschet J, Guillaume T, Garnier A, Eveillard M, Le Bourgeois A *et al.* FLT3 ligand plasma levels have no impact on outcomes after allotransplant in acute leukemia. *Cytokine* 2019; **120**: 85–87.