Disrupting autophagy in *FLT3*-mutant acute myeloid leukemia

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In the study by Zhang *et al.*, published in this issue of *Haematologica*,[1] the authors examine the mechanisms by which the multi-kinase inhibitor CG-806 kills *FLT3*-mutant acute myeloid leukemia (AML) cells, with an emphasis on modulation of autophagy and related endoplasmic reticulum stress-associated pathways. They report that in *FLT3*-mutant cells, exposure to FLT3 inhibitors e.g., sorafenib and quizartinib, elicits an autophagic response, operating, at least in part, through microenvironmental factor-mediated induction of Bruton tyrosine kinase (BTK). Using both pharmacological (e.g., BTK inhibitors) and genetic strategies (e.g., *Atg7* knockdown), they demonstrate that disruption of BTK-induced autophagic responses increases the lethal effects of FLT3 inhibitors. Building on this foundation, they investigated the antileukemic activity of a novel multi-kinase inhibitor, CG-086, which inhibits FLT3, BTK, and in addition, aurora kinase A (AURA). Notably, they found that CG-806 induced cell death in association with inhibition of these kinases, as well as by disrupting autophagic events. Interestingly, the authors observed that interference with autophagy was primarily effective in *FLT3*-mutant AML models, but less so in wildtype *FLT3* cells, raising the possibility that cytoprotective autophagy is selectively relevant for the former cells. Nevertheless, the ability of CG-806 to kill *FLT* wildtype cells could be attributed to the ability of this agent to interfere with AURA function, causing cells to die from a form of mitotic catastrophe. Importantly, the authors demonstrated that CG-086 was active in primary AML cells obtained from patients resistant to a FLT3 inhibitor (e.g., sorafenib), and was quite tolerable and effective in a *FLT3*-mutant patient-derived xenograft model. Collectively, these findings argue that CG-086 represents a potentially important addition to the therapeutic armamentarium for *FLT3*-mutant AML, and potentially other AML subtypes.

The involvement of autophagy in FLT3 inhibitor-associated resistance in AML is an interesting concept, and one with obvious translational potential. The authors have previously described this phenomenon,[2] focusing on genetic strategies (e.g., *Atg7* knockdown) to validate its role in cell death. Here, they emphasize the potential for circumventing resistance by disabling this process pharmacologically. It should be noted that autophagy is a highly complex process, and its effects on cell death may vary extensively with cell context. For example, while autophagy can be cytoprotective under some circumstances,[3] under other circumstances it can also be cytotoxic or cytostatic.[4] Targeting cytoprotective autophagy, for example by agents such as chloroquine, has been the subject of great interest,[5] and the authors demonstrated that chloroquine did indeed enhance the effectiveness of CG-806. The concept of disabling autophagy by dual strategies, such as targeting signaling pathways implicated in autophagy induction (e.g., FLT3 and BTK) as well as the autophagic apparatus directly (e.g., via chloroquine), warrants future consideration. It should be kept in mind that preventing the induction of autophagy, for example, by inhibiting signaling pathways may differ fundamentally from interfering with lysosome function, for example, by agents such as chloroquine.

The role of BTK, implicated in lymphomagenesis, in myeloid malignancies has previously been described,[6] including in *FLT*-mutant AML.[7] However, the mechanism by which inhibition of this signaling molecule induces AML cell death has not been identified. The results of the present study suggest that the actions of CG-806 may involve interruption of both BTK as well as microenvironmental factors, resulting in the prevention of cytoprotective autophagy. If validated in lymphoid malignancies, such findings could provide a foundation for employing this agent in the setting of non-Hodgkin lymphoma and potentially chronic lymphocytic leukemia.

It is interesting, but at the same time puzzling, that mechanisms responsible for the antileukemic effects of CG-086 appeared to be operative primarily in *FLT3*-mutant AML, but not in wildtype cells. Such findings raise the possibility that the ability of the former cells to mount a cytopro-

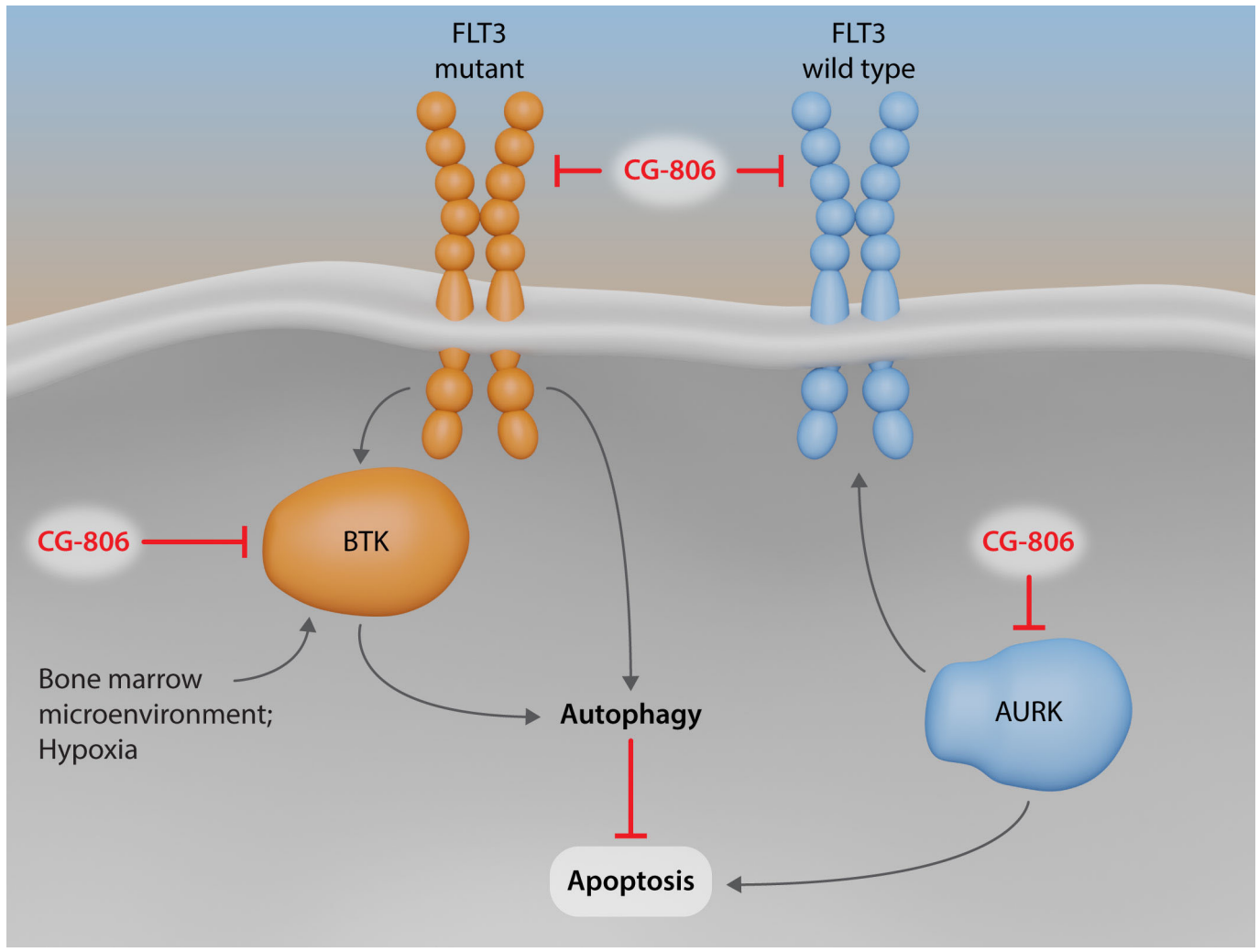

Figure 1. Mechanisms of CG-806 lethality in mutant and wildtype acute myeloid leukemia. In mutant *FLT3* disease, the lethal effects of inhibition of FLT3 are opposed by microenvironmental factor-mediated induction of cytoprotective autophagy operating through a BTK-dependent mechanism. In *FLT3* mutant cells, CG-806 inhibits both FLT3 as well as BTK, resulting in a marked increase in cell death. This process is not operative in wildtype *FLT3* acute myeloid leukemia, but such cells are killed by CG-806 through inhibition of FLT3 as well as disruption of aurora kinases (AURK), leading to death via G2M arrest and polyploidy. Thus, CG-806 is effective against both *FLT3*-mutant and wildtype disease.

tective autophagic response may contribute to the poor prognosis of this particular subtype of AML. Nevertheless, wildtype cells remained susceptible to CG-086, a phenomenon attributed to the ability of this agent to inhibit AURA, resulting in inappropriate G2M phase transition and induction of a form of mitotic catastrophe.[8] This observation argues that while highly selective kinase inhibitors offer the promise of diminished off-target effects, multi-kinase inhibitors, such as CG-086, which disrupt multiple survival pathways may provide countervailing advantages in certain settings.

The ultimate role that CG-086 will play in the treatment of *FLT3*-mutant AML remains to be determined, but early clinical results appear to be promising, and at the very least suggest that this agent is tolerable in humans. Whether it will prove superior to other FLT3 inhibitors remains to be established, as does its role in the treatment of wildtype disease. It should be kept in mind that while the contribution of FLT3 inhibitors in AML is now firmly established, it is unclear whether such approaches will eradicate leukemia stem cell-like cells, given that *FLT3* mutations can be relatively late-appearing genetic aberrations, and that elimination of FLT3-positive cells is not by itself a curative strategy. However, the multiple mechanisms of action of CG-086 may address this issue, and preclinical studies examining the effects of CG-086 on more primitive AML progenitors, such as stem cell-like cells, are likely to be informative. Another question to be addressed is which of the multiple mechanisms of action of CG-086, for example, inhibition of FLT3, BTK, AURA, microenvironmental factors, and/or autophagy, is/are primarily responsible for antileukemic activity. It would also be interesting to determine whether and to what extent common survival pathways downstream of FLT3 and BTK, for example, MAPK and AKT pathways, contribute to the actions of this agent. For example, FLT3 interruption may sub-optimally inhibit these pathways[9] whereas concomitant BTK disruption may enhance signaling blockade. Finally, the possibility that these actions may cooperate to trigger leukemic cell death is quite likely, and adds to the complexity. In any event, the present observations, along with early clinical findings, indicate that CG-086 represents an interesting new FLT3 and multi-kinase inhibitor that warrants further scrutiny. The results of ongoing clinical trials are eagerly awaited and should help to determine whether this agent deserves a place in the therapeutic armamentarium for *FLT3*-mutant AML.

Disclosures

No conflicts of interest to disclose.

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