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## To the editor:

### Liver therapy in anemia: a motion picture by William P. Murphy

On December 10, 1934, the Caroline Institute awarded that year's Nobel Prize in Physiology or Medicine to 3 American investigators: George R. Minot and William P. Murphy of the Harvard Medical School (Boston, MA) and George H. Whipple of the University of Rochester School of Medicine and Dentistry (Rochester, NY), "in recognition of their discoveries respecting liver therapy in anaemias." On December 12, 1934, Murphy delivered the Nobel Lecture and in the concluding paragraph stated, "Rather than enlarge further upon the details and results of the treatment of pernicious anemia, I shall now present, with your permission, a motion picture which will illustrate many points more clearly than I could discuss them here."<sup>1</sup> In this letter we present what we believe was the motion picture to which Murphy referred. The motion picture, made at the Peter Bent Brigham Hospital, emphasizes the superiority of parenteral liver extract to oral whole liver and liver extract in the treatment of pernicious anemia (PA). The movie (Movie S1, available at the *Blood* website; click on the Supplemental Movie link at the top of the online letter) was found in the Peter Bent Brigham Hospital and given to M.A.S., but the details of its rediscovery are unknown.

In 1900, Russell gave a full account of the spinal cord involvement in PA and coined the term "subacute combined degeneration of the spinal cord."<sup>2</sup> It was noted that hematologic abnormalities in patients with tropical sprue improved with a diet containing milk, meat, cod-liver oil, and oranges.<sup>3</sup> This observation led to successful use of similar treatments in patients with PA.<sup>3</sup> The hematopoietic properties of liver and meat were demonstrated by Whipple while working on dogs that had been bled to produce anemia.<sup>4</sup> Whipple demonstrated that the most effective dietary addition in chronic anemia was raw liver. Minot took detailed dietary histories from patients and noted that often his patients with PA excluded meat from their diets. Minot and Murphy started treating PA patients with liver. The diet recommended by Minot and Murphy consisted of 120 to 240 g cooked beef liver, 120 g or more of beef or mutton "muscle meat," and some vegetables, fruits,

eggs, and milk taken daily.<sup>5</sup> They documented improvement in the red blood cell count and a sharp rise in the reticulocyte count.<sup>6</sup>

The accompanying video is approximately 7 minutes in length and is divided into 2 parts. In Part 1, Murphy illustrates the hematologic and neurologic signs and symptoms in PA. This is followed by an illustration of normal hematopoiesis and the derangements seen in PA. The last segment of the first part compares therapy with whole liver, oral liver extract, and concentrated extract for intramuscular injection. A demonstration of the intramuscular injection technique is also provided. The second part shows improvement in the peripheral smear with liver therapy. Murphy graphically illustrates the brisk reticulocytosis, the lag in increase in red cell count, and the greater effectiveness of parenteral therapy compared with oral therapy. The latter section of Part 2 deals with cost-effectiveness of the parenteral therapy and the importance of maintenance therapy.

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*The online version of this letter contains a data supplement.*

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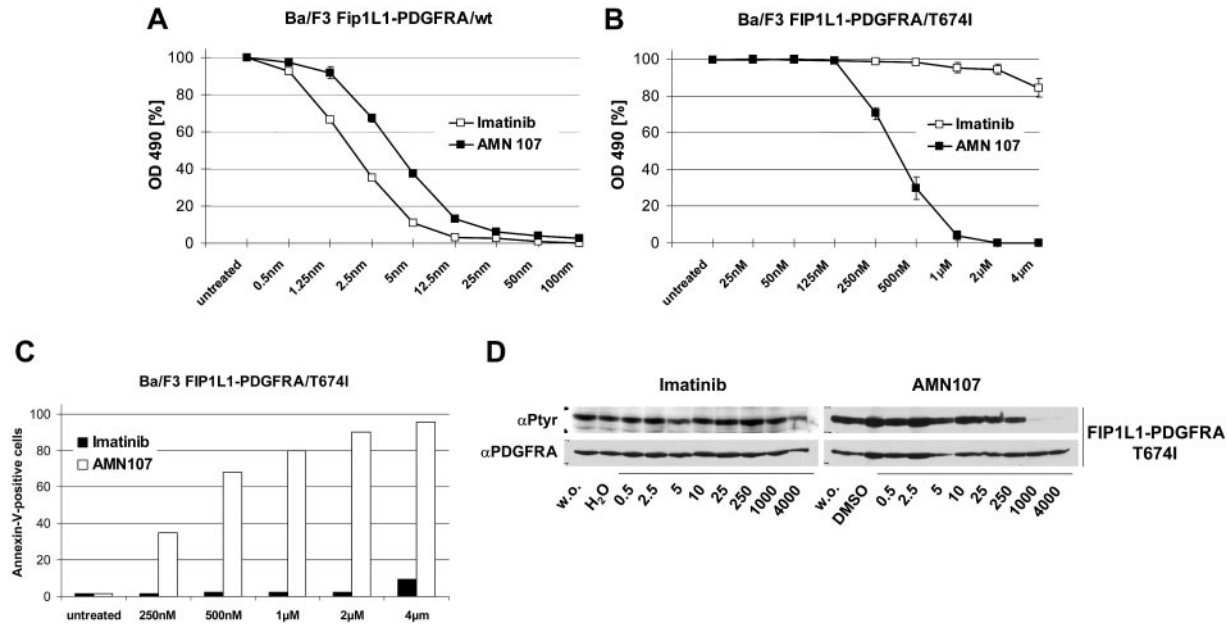
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## To the editor:

### The FIP1L1-PDGFRα T674I mutation can be inhibited by the tyrosine kinase inhibitor AMN107 (nilotinib)

A fusion of the *PDGFRα* and *FIP1L1* genes can be detected in cases of idiopathic hypereosinophilic syndrome (HES),<sup>1</sup> and the resulting tyrosine kinase constitutes a drug target for the treatment of this disease with the tyrosine kinase inhibitor imatinib mesylate

(Gleevec; Novartis, Basel, Switzerland).<sup>1,2</sup> However, a mutation leading to threonine residue 674 in the FIP1L1-PDGFRα kinase domain being replaced by isoleucine is known to give rise to resistance to imatinib mesylate in patients with HES.<sup>1,3</sup> This T674I



**Figure 1. FIP1L1-PDGFR/T674I can be inhibited by AMN107 (nilotinib) but not imatinib mesylate.** Growth inhibition of Ba/F3 cells expressing FIP1L1-PDGFR/wt (A), and Ba/F3 cells expressing FIP1L1-PDGFR/T674I (B) by imatinib mesylate and AMN107. Proliferation was measured using an MTT-based method by absorption of formazan at 490 nm. Measures were taken as triplicates after 48 hours of culture without and in the presence of inhibitor at the indicated concentrations. Values are expressed as mean values of growth inhibition from 3 independent experiments. For each experiment, measures were taken in triplicates. Bars indicate  $\pm$  SE. OD indicates optical density. Induction of apoptosis by AMN107 in Ba/F3 cells expressing FIP1L1-PDGFR/T674I (C). Annexin V-positive cells were measured after 48 hours of culture in the presence of AMN107 at the indicated concentrations. Two experiments were performed. Results of 1 representative experiment are shown. Inhibition of autophosphorylation in FIP1L1-PDGFR/T674I transfected Ba/F3 cells (D). Cells were incubated without and in the presence of imatinib mesylate (left panel) or AMN107 (right panel) at the indicated concentrations. Cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Blots were probed for phosphotyrosine and PDGFR.

exchange corresponds to T315I in BCR-ABL, which causes absolute resistance of chronic myelogenous leukemia (CML) to any available tyrosine kinase inhibitor, including imatinib mesylate,<sup>4</sup> and the novel kinase inhibitor AMN107 (nilotinib; Novartis).<sup>5</sup> To determine whether the T674I mutation in FIP1L1-PDGFR causes resistance to AMN107 we used Ba/F3 cells transfected with both wild-type (wt) and the T674I mutant form of FIP1L1-PDGFR. As expected, both inhibitors suppressed the growth of cells expressing FIP1L1-PDGFR/wt at low nanomolar concentrations (Figure 1A). However, whereas FIP1L1-PDGFR/T674I caused a complete resistance to imatinib mesylate, AMN107 was capable of suppressing the growth of Ba/F3 cells transfected with FIP1L1-PDGFR harboring this mutation with an inhibitory concentration at 50% (IC<sub>50</sub>) of 376 nM (Figure 1B). Also, AMN107, but not imatinib mesylate, induced apoptosis in FIP1L1-PDGFR/T674I cells (Figure 1C). This was accompanied by suppression of FIP1L1-PDGFR autophosphorylation in the presence of AMN107 (Figure 1D). Thus, AMN107 is active against FIP1L1-PDGFR/T674I, a mutant that causes the resistance of HES to imatinib mesylate. This finding is in contrast to a recent report by Stover et al,<sup>6</sup> describing resistance of FIP1L1-PDGFR/T674I to AMN107. The conflicting findings may in part be explained by lower AMN107 concentrations used in some experiments in the report by Stover et al. It has to be emphasized that in our experiments, complete suppression of FIP1L1-PDGFR/T674I was obtained at AMN107 concentrations that are achievable in patients where maximal and trough plasma drug concentrations of 3.6 and 1.7  $\mu$ M were measured following treatment with AMN107 at 400 mg twice daily.<sup>7,8</sup> As in patients with CML, where the corresponding mutation T315I constitutes the most frequent exchange leading to imatinib mesylate resistance, T674I may turn out to be a frequent cause of imatinib mesylate resistance in HES

and is so far the only mutation identified in single cases of HES with imatinib mesylate resistance. Based on the presented data in such cases, a switch to AMN107 seems to be warranted.

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## Response:

### The definition of resistance is in the eye of the beholder

We appreciate the observations made by Duyster et al that relate to sensitivity of clinical alleles of *FIP1L1-PDGFR*A associated with hypereosinophilic syndrome to inhibition with AMN107. Our cellular and biochemical experiments are generally in agreement in showing that the *FIP1L1-PDGFR*A T674I imatinib mesylate resistance allele is also highly resistant to inhibition with AMN107, with more than 100-fold higher IC<sub>50</sub> than for *FIP1L1-PDGFR*A.<sup>1</sup> There are several potential explanations for why we observed modest effects on cell growth and tyrosine phosphorylation content of *FIP1L1-PDGFR*A T674I transformed cells at 1 μM AMN107 compared with the results annotated here, but at most there may be a several fold difference in our estimates of cellular IC<sub>50</sub>.

Several other points merit consideration. First, we have previously reported that PKC412 is a potent inhibitor of the *FIP1L1-PDGFR*A T674I allele,<sup>2</sup> with an IC<sub>50</sub> of approximately 100 nM vs approximately 376 nM reported here for AMN107. From this perspective, PKC412 may be as appropriate an alternative for *FIP1L1-PDGFR*A patients who develop resistance due to acquired T674I as AMN107. In this context, it is also worth noting the importance of including controls for nonspecific or off-target toxicities for tyrosine kinase inhibitors such as AMN107 or PKC412, as they are selective rather than specific. Parental Ba/F3 cells would have been 1 such control in these experiments, but in our view the most compelling controls for nonspecific toxicity or off-target effects are kinase mutations that are selected for resistance to the kinase inhibitor in question. For example, we generated a PKC412 resistance allele in the context of *FIP1L1-PDGFR*A with an N659D substitution.<sup>2</sup> We observed that cells stably transduced with *FIP1L1-PDGFR*A N659D were resistant to the effects of PKC412, establishing *FIP1L1-PDGFR*A as the critical target for cellular cytotoxicity mediated by PKC412 in this context, rather than off-target or nonspecific effects. Indeed, the finding of the *FIP1L1-PDGFR*A T674I imatinib mesylate resistance mutation in a patient with clinically resistant HES provides the most compelling evidence that *FIP1L1-PDGFR*A causes HES and is the target of imatinib mesylate. It would be useful to have comparable controls for AMN107 as well, although it seems most likely that the cytotoxic effects of AMN107 treatment are attributable to inhibition of the T674I allele.

Second, we completely agree that relative resistance of *FIP1L1-PDGFR*A T674I to AMN107 does not exclude potential clinical applicability and efficacy if adequate plasma concentrations can be achieved. It may be of value to consider either AMN107 or PKC412 as second-line agents. For the time being, the point is

moot, as neither of these drugs is Food and Drug Administration (FDA) approved for any indication, but could potentially be accessed through compassionate use mechanisms.

It may be, as suggested by the authors, that *FIP1L1-PDGFR*A T674I will emerge as an important clinical problem in treatment of HES, but so far it has not. Over the last several years increasing numbers of patients with HES have been treated with imatinib mesylate, but there are only 2 reported cases of resistance to imatinib mesylate.<sup>3,4</sup> Both of these developed in the context of acute eosinophilic leukemias rather than in HES. It is certainly possible that more cases will ultimately surface, and proactive development of inhibitors such as PKC412 and AMN107 that address this problem seems a prudent course of action. But it is possible that these will be no more effective in the setting of acute eosinophilic leukemia than imatinib mesylate is in the context of CML in blast crisis.

However, it is also possible that resistance will develop less frequently in *FIP1L1-PDGFR*A-positive HES due to the remarkable potency of imatinib mesylate in this context. Imatinib mesylate is several hundred-fold more potent as a *FIP1L1-PDGFR*A inhibitor than as a BCR-ABL inhibitor. Only time will tell, but one might hope that highly potent kinase inhibitors such as imatinib mesylate in the context of *FIP1L1-PDGFR*A, or AMN107 or dasatinib in the context of BCR-ABL-positive disease, will more effectively suppress emergence of resistant clones due to point mutations in the target kinases, than lower potency inhibitors.

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## To the editor:

### Bevacizumab therapy for POEMS syndrome

We read the case report from Badros and coworkers with great interest.<sup>1</sup> A patient with POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) was successfully treated with bevacizumab. Increased

level of vascular-endothelial growth factor (VEGF) is thought to play a role in the pathophysiology of POEMS syndrome.<sup>2,3</sup> Here we describe a POEMS syndrome patient with high VEGF levels, treated with bevacizumab, without clinical improvement.