Supplementary Materials and Methods

Methods

Cellular TF activity assay

Cells were washed with PBS without calcium and magnesium (Thermo Fisher Scientific, Cat #14190-250), reconstituted with Hank's balanced salt solution without calcium and magnesium (Thermo Fisher Scientific, Cat #14170-112), and disrupted by sonication (total of ~24 000 J) using a Q700 sonicator (Qsonica, Newtown, CT, USA).

The clotting time was measured using a STart4 coagulation analyzer (Diagnostica Stago, Parsippany, NJ, USA). Briefly, 50 µL of sonicated cellular suspension was added to a mixture of 50 µL of 25 mM CaCl2 solution and 50 µL of human plasma (Pacific Hemostasis Coagulation Control Normal, Level 1, Thermo Fisher Scientific). TF activity was calculated using a standard curve generated by Innovin (Siemens, Munich, Germany). TF activity was normalized by protein concentration determined using a BCA assay.

Blood Collection and plasma preparation

For measurement of plasma proteins and circulating blood cells and associated biomarkers, such as mean platelet volume (MPV), sodium citrate was injected into the inferior vena cava of mice and blood was collected into syringes as described[1]. Platelet-poor plasma was prepared by centrifuging whole blood at 4,500 x g for 15 minutes at room temperature. Plasma was immediately frozen and stored at -80°C.

Blood smears and Giemsa staining

Blood smears were prepared on glass slides. After drying, the smears were fixed in methyl alcohol for 60 seconds. The slides were then soaked with Wright Giemsa solution (Fisher Scientific, CAT#23-264983) for 1 minute. After washing with pH 6.4 PBS for 1 minute with rocking, another 2 minutes incubation without rocking. The slides were rinsed with deionized water and allowed to air dry. Blast cells and leukocytes were counted to 100 and percentage of the blast cells were calculated.

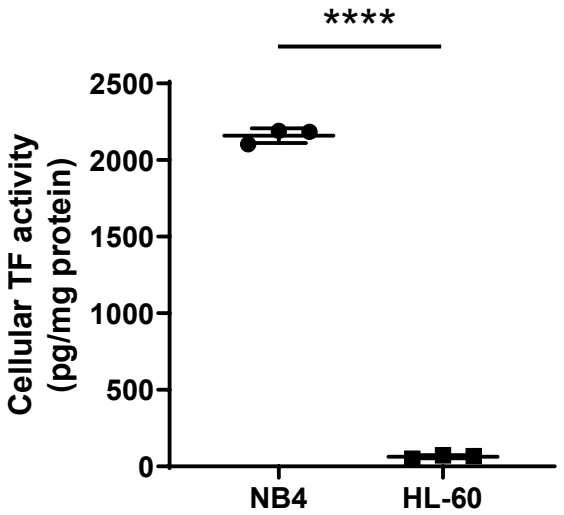
Intracranial bleeding model

We used an intracranial bleeding model that was described previously[2]. Mice were anesthetized with 3% isoflurane and then positioned prone in a stereotaxic head frame (Stoelting Co. Wood Dale, IL, USA, CAT#51730) while maintaining 3% isoflurane. A scalp incision was made along the midline and a burr hole (1 mm) was drilled on the left side of the skull (0.2 mm anterior and 2.0 mm lateral of the bregma). A 26-gauge needle attached to a micro syringe (Hamilton Company, Reno, NV, USA, CAT#87930) was inserted into the brain through the burr hole with stereotaxic guidance (stereotaxic coordinates: 2.0 mm lateral to the midline, 0.2 mm anterior to the bregma, and 3.7 mm below the skull), and 1 μL (0.025 Unit/μL) of bacterial collagenase (Sigma Aldrich, St. Louis, MO, USA, CAT#C0773) solution was injected at a rate of 0.5 μL/min for 2 minutes, with the needle left in place for an additional 5 minutes after injection. Three (xenograft model) or twenty-four (allograft model) hours later, mice were perfused with 20mL PBS and 20 mL 4% paraformaldehyde. Then, brains were removed, cut in 2-mm thick coronal sections, and photographed to visualized bleeding. Lesion area was quantified using Image J software (version 1.53t, National Institute of Health, Bethesda, MD, USA). The volume of the lesion was calculated by multiplying the thickness by the sum of the lesion areas of each

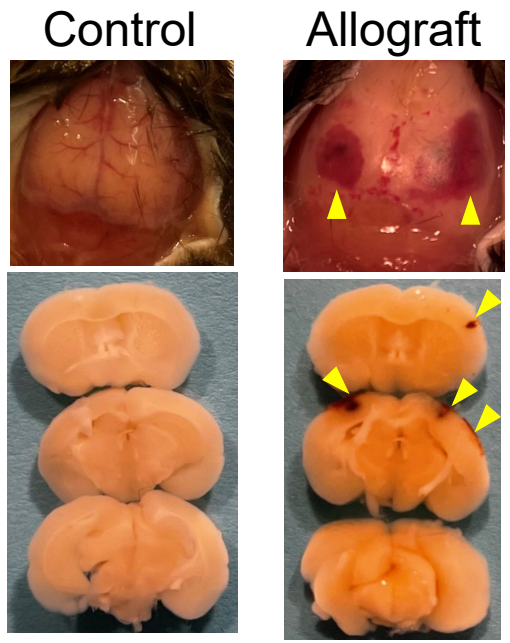
section[3]. The xenograft and allograft models were subjected to intracranial bleeding on days 28 and 21, respectively.

References:

1. Sommeijer DW, van Oerle R, Reitsma PH, et al. Analysis of blood coagulation in mice: pre-analytical conditions and evaluation of a home-made assay for thrombin-antithrombin complexes. *Thromb J*. 2005;3:12.
2. Wang S, Reeves B, Sparkenbaugh EM, et al. Protective and detrimental effects of neuroectodermal cell-derived tissue factor in mouse models of stroke. *JCI Insight*. 2016;1(11).
3. Wang J, Rogove AD, Tsirka AE, Tsirka SE. Protective role of tuftsin fragment 1-3 in an animal model of intracerebral hemorrhage. *Ann Neurol*. 2003;54(5):655-664.



Supplementary figure 1



Supplementary Figure 2

Supplementary Figure 1. Cellular TF activity in NB4 cells and HL-60 cells. Cellular TF activity of NB4 cells and HL-60 cells were shown. Data are shown as mean ± SD. The unpaired two-tailed Student's t-test was used. ***$P < 0.0001$.

Supplementary Figure 2. Spontaneous intracranial hemorrhage in a mouse in the allograft model. One of two mice in the allograft model that died on day 24 showed spontaneous intracranial bleeding at autopsy. Yellow arrows indicate the area of the bleed. The upper panels are brain slices, and the lower panels are gross pictures of the skulls of control and leukemic mice.