before morning drugs and PD 0360324 infusion). Monocytes were immediately stained, analyzed on FACSCanto flow cytometer (BD Biosciences, Immunocytometry Systems), and enumerated as previously described.[8] After study closeout, trial unblinding revealed that the female patient received the anti–M-CSF mAbs and the male received placebo.

The number of classical monocytes did not differ between patients. Mean numbers ± SD in antibody-treated patient versus placebo-treated patient were: 530 ± 216 vs 558 ± 104 cells/μL (Figure 1A). However, the nonclassical monocytes disappeared in the female patient after anti–M-CSF mAb infusions (Figure 1C), which was best noticed (for at least 1 week) after 1st and 2nd dosing. Four weeks after each dosing the number of nonclassical monocytes was approaching baseline level and achieved it on day 112. These changes were not observed in the patient on placebo. Similar changes were seen in intermediate monocytes (Figure 1B); however, after infusions their number recovered earlier than that of nonclassical monocytes. This supports previous suggestions that steady-state plasma levels of M-CSF promote differentiation of classical monocytes to intermediate and nonclassical monocytes, and that this process may be inhibited by neutralizing anti–M-CSF mAbs. This is visualized on the dot plots showing dynamic changes of monocyte subpopulations during the first 4 weeks after initial anti–M-CSF mAb infusion in the female patient (Figure 1D). Our data provide, for the first time, direct evidence that M-CSF is essential for differentiation of intermediate and nonclassical monocytes in vivo.

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To the editor:

Programmed vesicle transfer of green fluorescent protein from a stably transduced cell line to primary hematopoietic cells

Release and trafficking of cell membrane–derived vesicles is a constitutive cellular function important to cell-cell communication.[1] Montecalvo and colleagues recently described the transfer of microRNA (miRNA) between dendritic cells.[2,3] The authors report the selective incorporation of specific miRNA in vesicle compartments as a function of cellular-differentiation stage and demonstrate endogenous miRNA transfer to bystander cells via nanosized vesicles with translational suppression of cognate mRNA targets. Even as the molecular mechanisms for protein or RNA incorporation into vesicles remain to be clarified, these elegant studies confirm earlier work demonstrating cell-cell transfer of coding and noncoding small RNA via vesicles with modulation of the target cell phenotype.[1,4,5] At the same time they leave open to what extent vesicle trafficking occurs between nonimmune cells and whether it can be exploited for the programmed transfer of nonendogenous protein or RNA.

To test the hypothesis that protein or coding RNA can be deliberately transferred between nonimmune cells via vesicles, we genetically modified Jurkat T cells with a vesicular stomatitis virus G protein–pseudotyped lentivector bearing a green fluorescent protein expression cassette (Jurkat-GFP). Vesicle release by Jurkat cells has recently been studied by others and our transmission

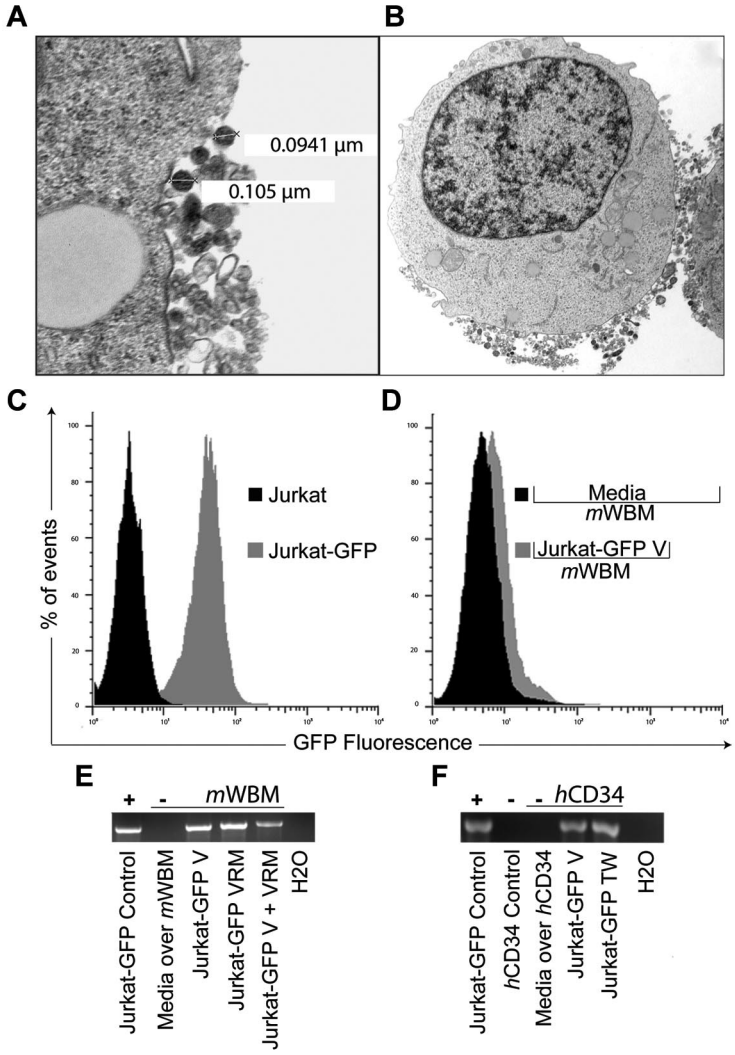

Figure 1. GFP expression and mRNA transfer via exosome trafficking between non-immune cells. (A) Electron micrograph of vesicle release from a Jurkat cell. Cell preparations on UV activated carbon formvar 400 Mesh copper grids (Ted Pella 01 822-F), were imaged at 100 kV on a Philips CM120 TEM microscope. Images were collected on a Gatan 794CCD multiscan camera and converted into 8-bit gray-scale TIF, 28 000× magnification. OHSU-Electron Microscopy Resource (B) Jurkat cell with representative, exosome-sized, vesicles located at the limiting membrane at 7100× magnification. (C) Histogram representation of green fluorescent protein (GFP) expression by Jurkat cells (Jurkat-GFP) after replication deficient retrovirus vector transduction (vesicular stomatitis virus G protein pseudotype, MOI 1, GFP expression cassette) and non-transduced control (Jurkat). (D) Overlay histogram demonstrating GFP expression in murine whole bone marrow cells (*m*WBM) 48 hours after transwell (0.4 μm pore) exposure to Jurkat-GFP derived vesicles (Jurkat-GFP-V) or media control (Media over *m*WBM). Vesicles (V) were isolated from culture media by differential centrifugation at 300*g* × 10 minutes, 2 000*g* × 15 minutes, 10 000*g* × 20 minutes, and at 100 000*g* × 2 hours. The pellet was washed, ultracentrifuged at 100 000*g* for 2 hours and resuspended in PBS. (E) Reverse transcription PCR analysis indicating the presence or absence of GFP sequence in *m*WBM cells after indicated coculture conditions: 48-hour transwell (TW), concentrated vesicles (V) or vesicle rich media (VRM) from Jurkat-GFP cells versus media control. RNA was extracted using RNeasy (QIAGEN). Complementary DNA was synthesized using the SuperScript III First Strand Synthesis Kit (Invitrogen) with oligo-dT priming followed by PCR. (F) Detection of GFP transcripts in non-mobilized normal human CD34$^+$ (*h*CD34) cells (Stem Cell Technologies). Culture condition, sample handling and reverse transcription PCR analysis as in panel E. Experiments were repeated with similar results.

electron microcopy studies (Figure 1A) confirm that these were predominantly in the exosome size range (30-110 nm; Figure 1B). Jurkat-GFP cells were propagated in bulk, enriched by flow-cytometric sorting for $> 90\%$ purity, with stable proviral integration indicated by GFP expression over serial (> 20) passages (Figure 1C). Twenty-four-hour culture supernatant from Jurkat-GFP and parental Jurkat cells was collected and processed to generate cell-free vesicle preparations by standard sequential gradient centrifugation.[6] To demonstrate successful transfer we exposed murine bone marrow cells (*m*WBM, C57B/6 strain) to Jurkat-GFP–derived purified vesicles (Jurkat-GFP-V) for 48 hours and detected GFP expression in *m*WBM by flow cytometry (Figure 1D). We tested vesicle transfer in 2 additional cell-cell contact-independent settings: as vesicle rich media (VRM) after removing debris from the harvest culture supernatant (2000*g* × 15 minutes) and in 0.4-μm transwell coculture (TW). After 48-hour coculture we observed successful amplification of GFP transcripts in DNAse-treated RNA from *m*WBM cells under all 3 conditions (Figure 1E). To highlight the potential relevance of our findings, these studies were repeated using human CD34$^+$ (hCD34; StemCell Technologies) target cells, with identical results, showing cell contact–independent vesicle transfer of GFP transcript from Jurkat-GFP cells (Figure 1F).

Taken together, our experiments demonstrate the cell-cell contact-independent transfer of nonendogenous coding RNA from stably transduced Jurkat cells to murine and human hematopoietic targets after purified vesicle, vesicle-rich media, or transwell exposure. As a caveat, these observations do not yet resolve to what extent GFP protein transfer, versus mRNA translation, accounts for the observed expression in hematopoietic target cells. Notwithstanding, our report extends the observations by Montecalvo and colleagues by revealing the feasibility of vesicle trafficking for the programmed transfer of stably introduced transgenes between nonimmune cells. We believe the deliberate cell-cell vesicle transfer of nonendogenous RNA may be useful for the experimental, and potentially therapeutic, manipulation of hematopoietic and other stem cells.

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To the editor:

Absence of *BRAF*-V600E in the human cell lines BONNA-12, ESKOL, HAIR-M, and HC-1 questions their origin from hairy cell leukemia

Hairy cell leukemia (HCL) shows distinct clinicopathologic, immunophenotypic, and gene expression features.[1-3] We previously identified the *BRAF*-V600E mutation as the disease-defining genetic event in HCL.[4] This mutation is present in virtually all cases of HCL but rarely in other B-cell lymphomas, remains stable over time (being consistently detectable at relapse), and leads to the constitutive activation of the mitogen–activated protein kinase (MAPK) pathway[4] that is potentially druggable with *BRAF*-V600E inhibitors. These findings have been confirmed in 2 studies recently published in this journal.[5,6]

Functional assays in HCL have been hampered by the scarcity of leukemic cells available for analysis because of frequent

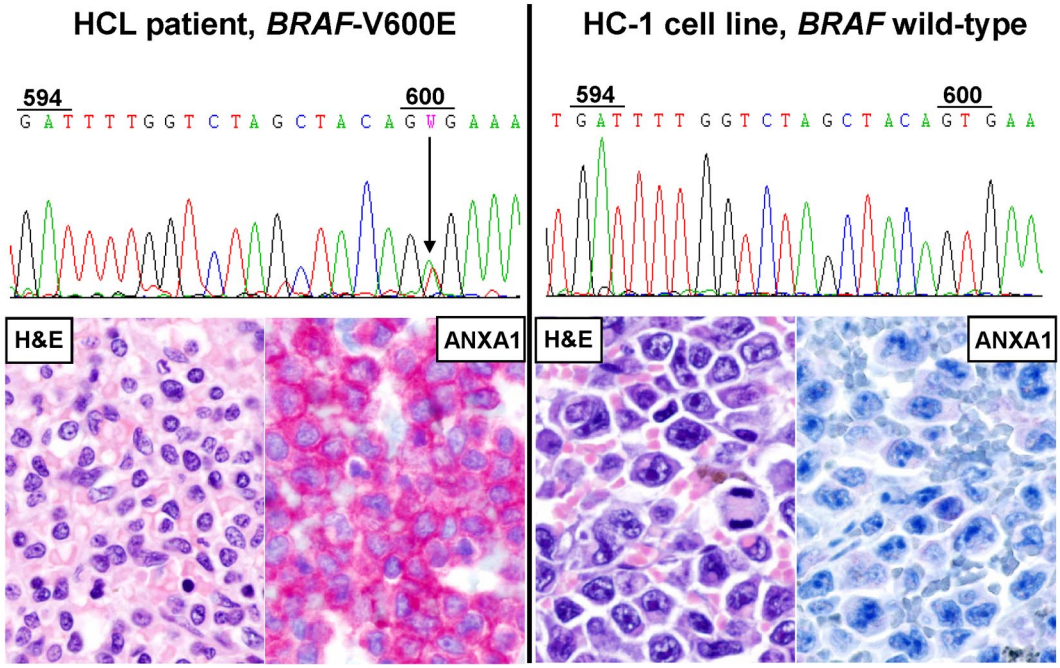

Figure 1. Human cell lines of putative HCL origin lack the *BRAF*-V600E mutation and key phenotypic features of HCL. Direct DNA Sanger sequencing of *BRAF*-exon15[4] in the HC-1 cell line (right chromatogram) shows the absence of the T-to-A point mutation at codon 600 leading to the V600E amino acid replacement, which is instead present heterozygously in primary leukemic cells MACS-purified from the peripheral blood of an HCL patient (left chromatogram). Both HC-1 cells and patient's leukemic cells display a wild-type codon 594 (GAT), as opposed to HAIR-M cells harboring a clonal heterozygous T-to-A point mutation at this codon (not shown) that leads to the D594E amino acid replacement. HC-1 cells xenografted in an NSG mouse show diffuse infiltration of the spleen by large B-cell lymphoma-like cells (right H&E staining) that are negative for annexin-1 (ANXA1; right ANXA1 immunostaining[1]). Conversely, the splenectomy specimen of the HCL patient is infiltrated by small mature-looking lymphoid cells with wide pale cytoplasm (left H&E staining) strongly expressing annexin-1 (left ANXA1-staining). All micrographs were collected using an Olympus B61 microscope (equipped with an Olympus UPlanApo 40×/0.8 NA objective and with an Olympus E330-ADU1.2x camera) and were acquired and processed using Olympus cell^B imaging software.