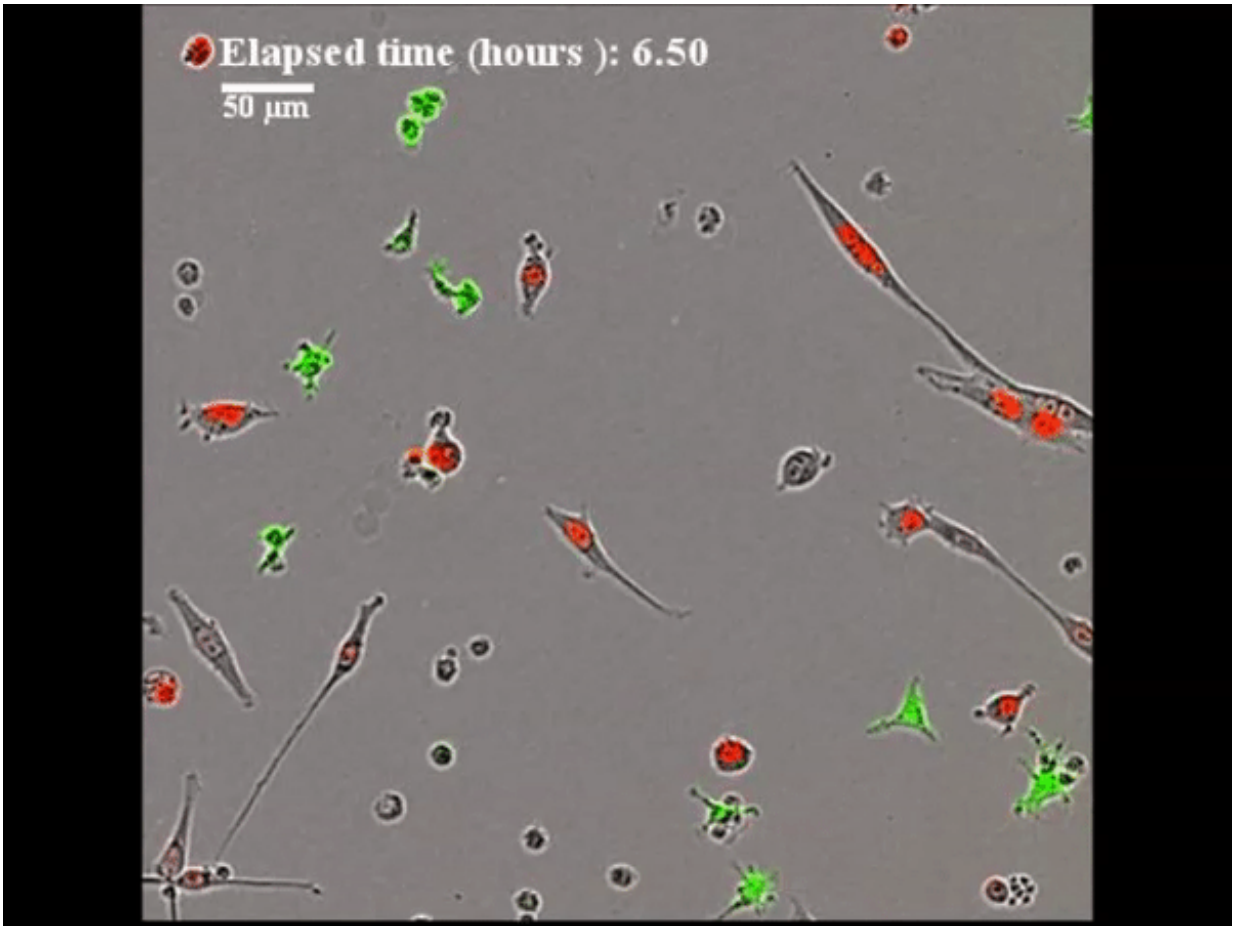
Fusion hybrids: A newly discovered population of tumor cells

September 24 2018, by Thamarasee Jeewandara



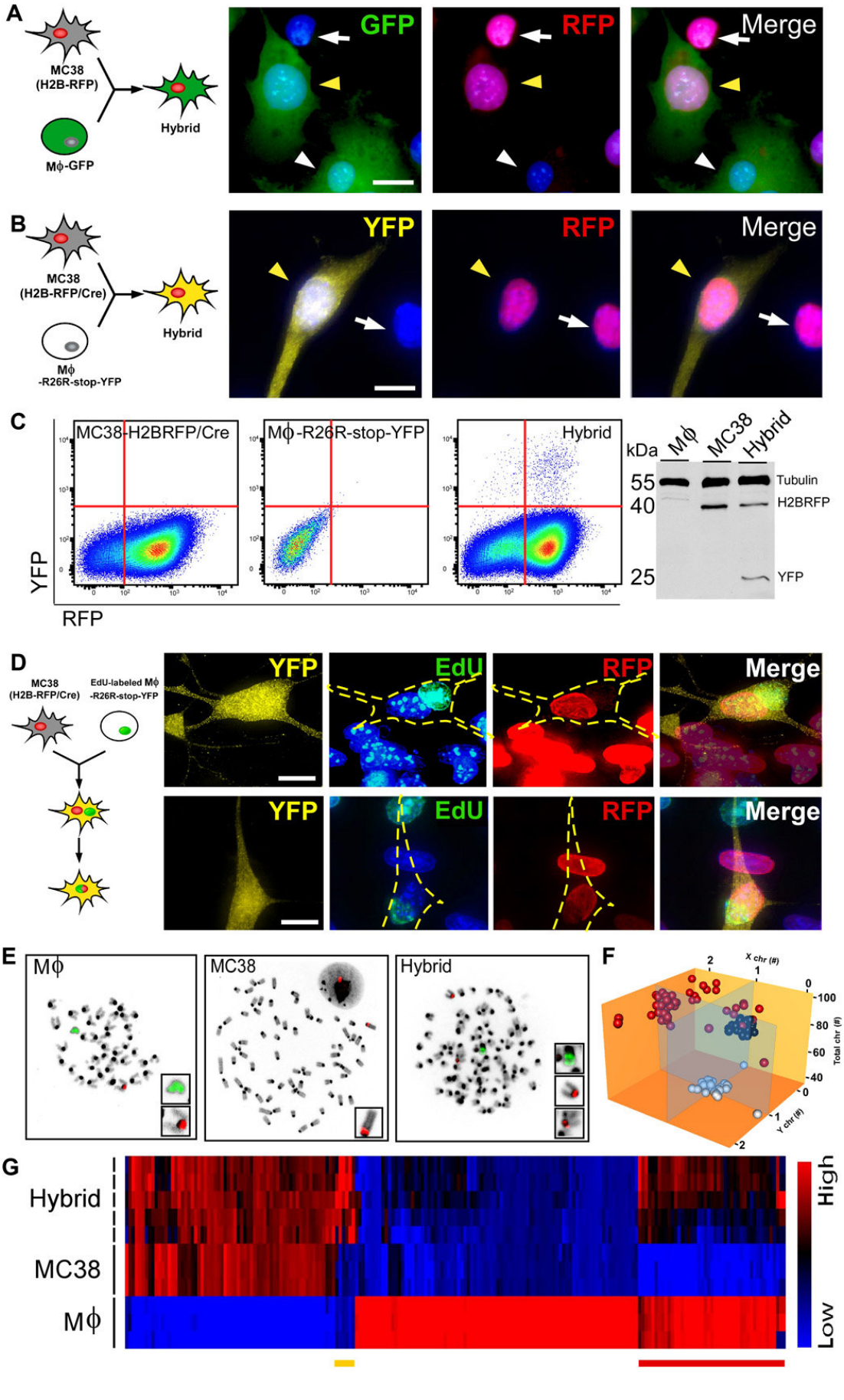
Engineering MΦ-cancer cell fusion hybrids. Identifying MΦ-cancer cell fusion hybrids via the co-expression of nuclear red fluorescent protein (RFP) and cytoplasmic green fluorescent protein (GFP) or yellow fluorescent protein (YFP). Credit: Science Advances, doi: 10.1126/sciadv.aat7828

In a recent study published in *Science Advances*, Charles E. Gast and co-workers detail the spontaneous process of cancer cell fusion with white blood cells to produce heterogenous hybrid clones in multiple biological systems, including in mice and in humans. The authors identified a new population of tumor cells that may reveal insight into a potential therapeutic target for intervention in human cancer. Despite a century-old hypothesis that cell fusion contributes to tumor initiation and metastatic behaviour acquisition, only a few experimental studies have addressed the functional underpinnings of cell fusion in the etiology of malignant progression. Preceding biological studies have only circumstantially addressed the significance of hybrid tumor cells to support the mechanism. The underlying intracellular mechanisms cannot be easily identified or determined in human subjects, therefore, murine models and in vitro studies have provided an appropriate platform in the present study.

Previous work by the same research group reported in vivo fusion between the intestinal epithelial cells and macrophages (also referred to as MΦs) in a cancer context. The results generated hybrid offspring that retained epithelial character as defined by their gene expression profile. As a follow-up, the authors presented a systematic analysis of MΦ–neoplastic cell fusion (referred to as MΦ–cancer cell fusion or fusion hybrid) with ex vivo and in vivo murine cancer models. The study provided evidence that hybrids acquired functional MΦ-associated phenotypes to enhance tumor progression. Subsequent analysis of human tumor biopsies and peripheral blood revealed a novel circulating hybrid cell (CHC) population. These cells were defined to harbor hematopoietic and epithelial/tumor properties. The number of hybrid cells correlated with the disease stage and predicted the overall outcome, providing a biomarker for patient classification.

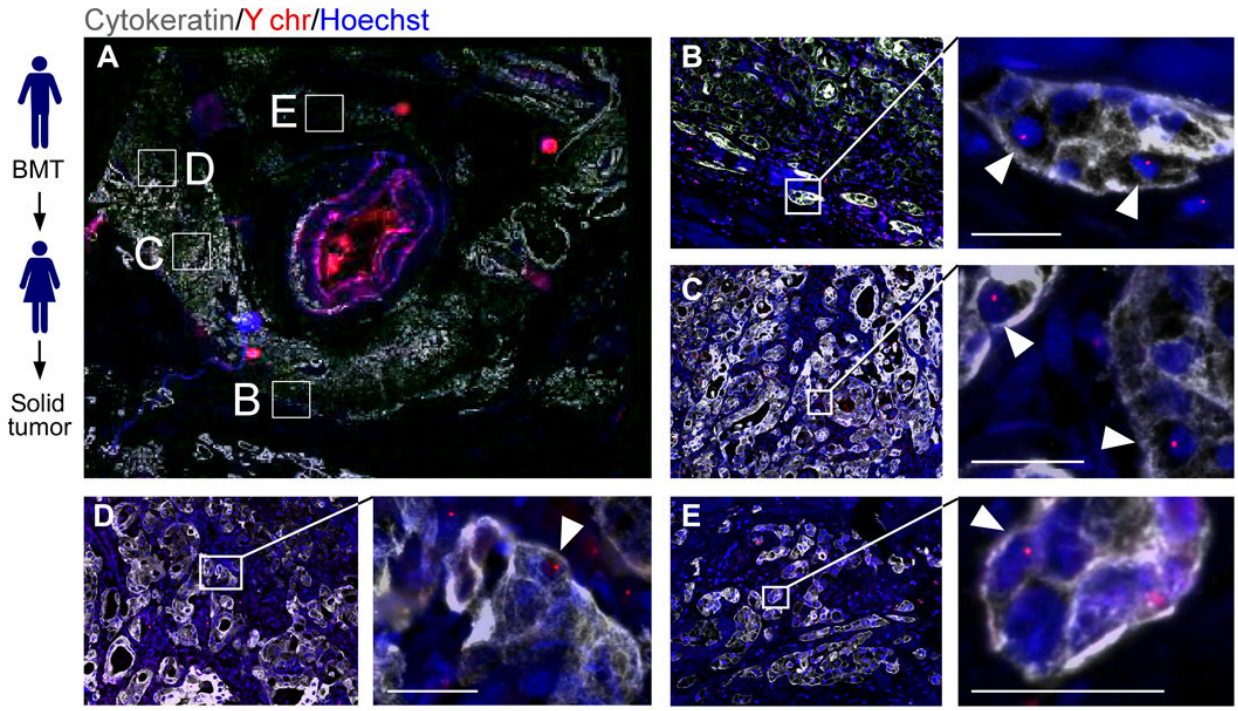
In the study, the authors generated in vitro-derived hybrids by engineering two mouse cancer cell lines: colon adenocarcinoma and

melanoma (MC38 and B16F10). The results indicated that MΦ-neoplastic cell fusion resulted in a variety of hybrid cells that shared features of both parental predecessors, while retaining their own characteristics.


A
MC38
(H2B-RFP)
Hybrid
Mϕ-GFP
GFP
RFP
Merge
B
MC38
(H2B-RFP/Cre)
Hybrid
Mϕ
-R26R-stop-YFP
YFP
RFP
Merge
C
MC38-H2BRFP/Cre
Mϕ-R26R-stop-YFP
Hybrid
YFP
RFP
kDa
Mϕ
MC38
Hybrid
55
40
25
Tubulin
H2BRFP
YFP
D
MC38
(H2B-RFP/Cre)
EdU-labeled Mϕ
-R26R-stop-YFP
YFP
EdU
RFP
Merge
E
Mϕ
MC38
Hybrid
F
X chr (#)
Y chr (#)
Total chr (#)
G
Hybrid
MC38
Mϕ
High
Low

B16F10 in vivo derived fusion hybrids in mice. A) Recipient mice injected intradermally with B16F10 melanoma cells readily developed 1.0 cm fusion tumors. B) Subsequent fluorescent analysis revealed fusion hybrids (red - RFP and green - GFP). C) To determine if hybrids retained tumorigenicity 300-fluorescent activated cell sorting (FACS)-isolated in vivo-derived hybrid cells were injected intradermally into secondary recipient mice. D) Representative FACS plot: hybrids represented a rare neoplastic cell population within the primary tumor. E) The resultant tumor growth indicated that hybrid cells retained tumorigenesis. F) To assess tumor heterogeneity 3000 in vivo-derived fusion hybrids were injected into 3 mice, the fusion hybrids displayed different rates of tumor growth. G) Macroscopic view of lungs and H&E view of a tissue section. Metastatic tumor area showed markedly greater burden than injected unfused tumor cells. H) Primary tumors were dissociated into single cells and fusion hybrids co-expressing RFP and GFP analyzed for cell surface MΦ-antigen expression. I) To establish MΦ as a fusion partner, B16F10 cells were injected into transgenic mice. Analyses of the primary tumor and lung metastases revealed microphthalmia-associated transcription factor (MITF)-expressing tumor cells (red) with GFP expression (green). Credit: *Science Advances*, doi: 10.1126/sciadv.aat7828

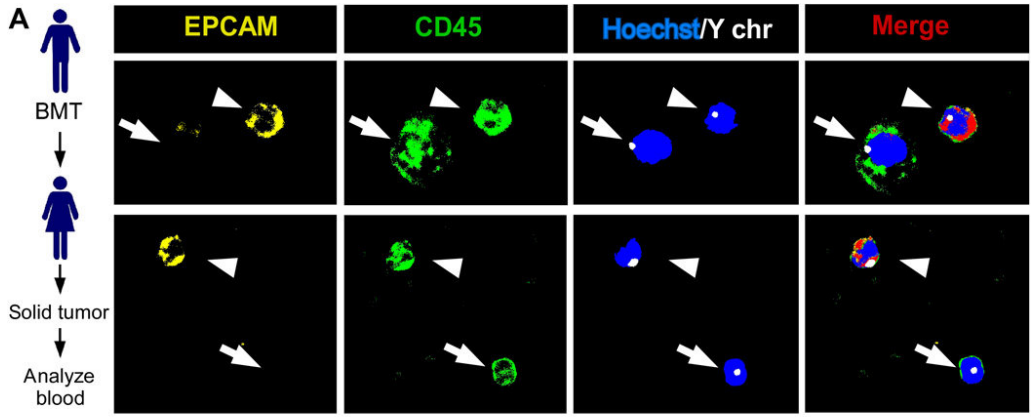
Next, the researchers studied cell fusion in mouse models of cancer after injecting 3000 in vivo-derived fusion hybrids into three mice. The results indicated that fusion hybrids showed different rates of tumor growth, suggesting the unique growth ability of hybrid cells and the ability to result in different rates of tumor growth.

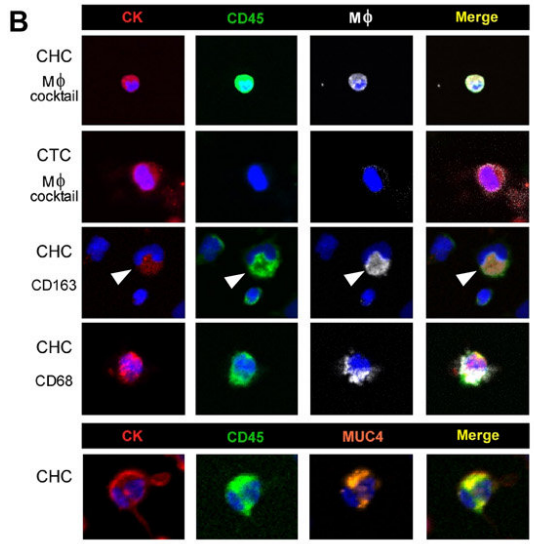


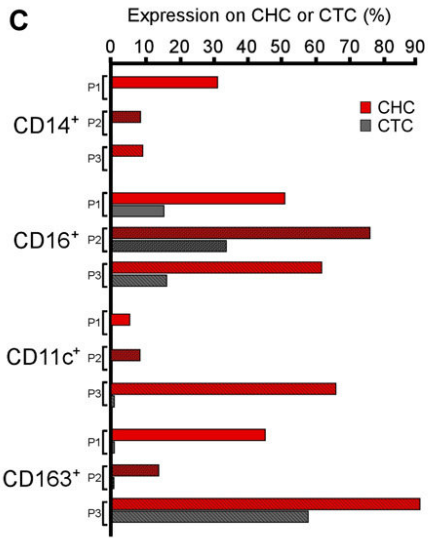
Cell fusion in humans – solid tumors from women with previous sex-matched bone marrow transplantation (BMT) permits analysis of cell fusion. A) Pancreatic ductal adenocarcinoma (PDAC)-tumor section with cytokeratin (gray) and Y chromosome (Y chr, red) and Hoechst (blue) staining revealed areas of cytokeratin-positive cells with Y-chromosome positive cells (white arrow heads). B) to E) Boxed representative areas are enlarged in (B) to (E). Credit: *Science Advances*, doi: 10.1126/sciadv.aat7828.

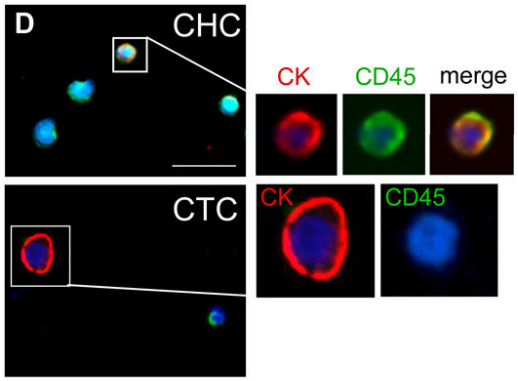
Gast and co-workers then analyzed tumor biopsies and peripheral blood from several female cancer patients to understand the biological significance of hybrids in humans. The researchers first determined if hybrids between blood cells and epithelial-derived cancer cells were detectable. The patients had previously received a sex-matched bone-marrow transplant that led to a second solid tumor, providing a disease scenario that supports the identification of hybrids harboring properties of blood cells and epithelial cells. The results indicated that all biopsies

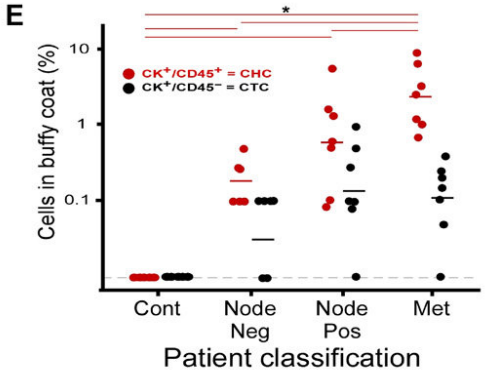
contained evidence of MΦ-tumor cell hybrids that correlated with the disease stage and rate of patient survival.

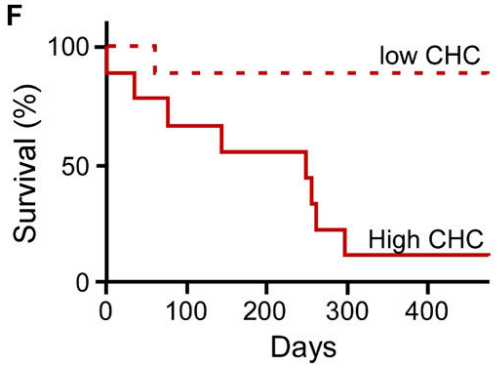

A
EPCAM
CD45
Hoechst/Y chr
Merge
BMT
Solid tumor
Analyze blood

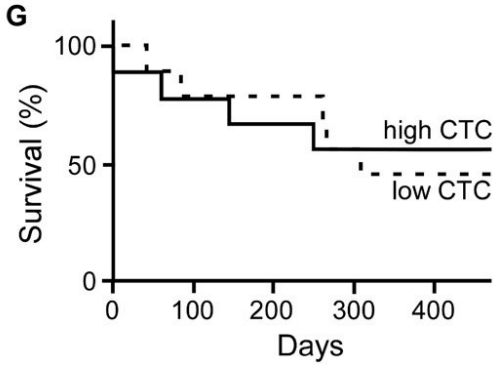

B
CK
CD45
Mϕ
Merge
CHC
Mϕ cocktail
CTC
Mϕ cocktail
CHC
CD163
CHC
CD68
CK
CD45
MUC4
Merge
CHC


C
Expression on CHC or CTC (%)
0 10 20 30 40 50 60 70 80 90
CHC
CTC
CD14+
CD16+
CD11c+
CD163+
P1
P2
P3


D
CHC
CK
CD45
merge
CTC
CK
CD45


E
*
CK+/CD45+ = CHC
CK+/CD45− = CTC
Cells in buffy coat (%)
10
1
0.1
Cont
Node Neg
Node Pos
Met
Patient classification


F
100
50
0
Survival (%)
low CHC
High CHC
0
100
200
300
400
Days


G
100
50
0
Survival (%)
high CTC
low CTC
0
100
200
300
400
Days

Human circulating tumor cells (CTCs). A) Circulating hybrid cells (CHCs) that co-expressed CD45 (a pan-leukocyte marker, green) and EPCAM (an epithelial marker, yellow) have a Y chromosome (white dot) in their nuclei (blue). Arrows show leukocytes. B) Determining the expression of MΦ via immunohistochemistry. C) Identifying MΦ epitope expression in CHCs and CTCs using flow cytometry. D) Digital image analyses to validate double-positive expression of CD45+ (green) and Cytokeratin+ (CK, red). E) The CK+/CD45+ and CK+/CD45- cells were quantified in patient blood across cancer stages. The number of CHCs expressing CK+/CD45+ significantly correlated with advanced disease. F and G) Kaplan-Meier curve associated with significantly increased risk of death for CHCs. Credit: *Science Advances*, doi: 10.1126/sciadv.aat7828.

The findings identified a novel population of tumor cells in circulation (CHCs) that were previously overlooked and excluded from routine analysis. The findings introduce a functionally significant aspect of tumor progression and evolution to uncover a new area of tumor cell biology. As a chimera of MΦs and neoplastic cells, the unique tumor population showed immune privilege in peripheral blood—a trait bestowed by their leukocyte identity. Understanding how such hybrids respond to immune therapies will offer an important area of investigation in the future. Together, the in vitro and in vivo data may reveal insight into diverse tumor cell pathophysiology underlying the treatment resistance, tumor progression and recurrence post-treatment in human cancer.

More information: Charles E. Gast et al. Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival, *Science Advances* (2018). DOI: 10.1126/sciadv.aat7828

Anne E. Powell et al. Fusion between Intestinal Epithelial Cells and Macrophages in a Cancer Context Results in Nuclear Reprogramming, *Cancer Research* (2011). DOI: 10.1158/0008-5472.CAN-10-3223

Naohiro Terada et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion, *Nature* (2002). DOI: 10.1038/nature730