The strategic combination of trastuzumab emtansine with oncolytic rhabdoviruses leads to therapeutic synergy

Rozanne Arulanandam1, Zaid Taha1,2, Vanessa Garcia1,2, Mohammed Selman1, Andrew Chen1, Oliver Varette1, Anna Jirovec1,2, Keara Sutherland1,2, Elizabeth Macdonald1,3, Fanny Tzelepis1, Harsimrat Birdi1,2, Nouf Alluqmani1,2, Anne Landry1, Anabel Bergeron1,2, Barbara Vanderhyden1,3 & Jean-Simon Diallo1,2✉

We have demonstrated that microtubule destabilizing agents (MDAs) can sensitize tumors to oncolytic vesicular stomatitis virus (VSVΔ51) in various preclinical models of cancer. The clinically approved T-DM1 (Kadcyla®) is an antibody-drug conjugate consisting of HER2-targeting trastuzumab linked to the potent MDA and maytansine derivative DM1. We reveal that combining T-DM1 with VSVΔ51 leads to increased viral spread and tumor killing in trastuzumab-binding, VSVΔ51-resistant cancer cells. In vivo, co-treatment of VSVΔ51 and T-DM1 increased overall survival in HER2-overexpressing, but trastuzumab-refractory, JIMT1 human breast cancer xenografts compared to monotherapies. Furthermore, viral spread in cultured HER2+ human ovarian cancer patient-derived ascites samples was enhanced by the combination of VSVΔ51 and T-DM1. Our data using the clinically approved Kadcyla® in combination with VSVΔ51 demonstrates proof of concept that targeted delivery of a viral-sensitizing molecule using an antibody-drug conjugate can enhance oncolytic virus activity and provides rationale for translation of this approach.

1 Centre for Innovative Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, ON K1H 8L6, Canada. 2 Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada. 3 Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada. ✉email: jsdiallo@ohri.ca

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**Results**

**T-DM1 improves VSVΔ51 oncolysis in resistant cancer cells.**

Owing to their potent cytotoxicity, tubulin inhibitors have been a mainstay for cancer therapy and are the most validated payloads for ADC-mediated targeted therapies. Conjugation of highly potent MDAs to antibodies can vastly improve their therapeutic window and tumor specificity. Trastuzumab emtansine (T-DM1) allows delivery of the otherwise toxic DM1 (maytansine derivative) specifically to HER2-overexpressing cells. T-DM1 has been clinically approved for the treatment of metastatic breast cancers that have previously been treated with trastuzumab and a taxane chemotherapy. Given that microtubule inhibitors can potentiate cancer-specific spread and oncolysis mediated by VSVΔ51, we sought to examine the combination of T-DM1 with VSVΔ51. As a starting point, we used VSVΔ51-resistant, interferon-responsive 786-0 renal carcinoma cells, a well-characterized system in our lab for assessing the impact of viral sensitizers. 786-0 cells also express detectable levels of HER2, and are able to bind Trastuzumab (Supplementary Figs. 1 and 2). 786-0 cells were treated with increasing concentrations of T-DM1, and subsequently infected with VSVΔ51 encoding either GFP or luciferase, at a low multiplicity of infection (MOI 0.01–0.1). After 24 h, viral spread was followed by transgene expression using fluorescence microscopy to detect or quantify GFP (Fig. 1a and Supplementary Fig. 3). Viral titers were first estimated by comparing luciferase gene expression to that of a standard curve to compute viral expression units (VEU, Fig. 1b) as previously described. A significant, dose dependent increase in VSVΔ51 output was observed with T-DM1 at concentrations >25 µg/ml and peaking at 500 µg/ml (Fig. 1b). This observation was confirmed by standard plaque assay, and the observed increase in viral titer was found to be significant over mock upon treatment of 786-0 cells with either T-DM1 or the control MDA colchicine, while trastuzumab alone had no positive impact (Fig. 1c). The boost in viral titers with T-DM1 also correlated with a disruption of the microtubule network in 786-0 cells as observed by immunofluorescence staining for β-tubulin and an increase in the number of polynucleated cells from 7% (with T-DM1 alone) to 33% (Fig. 1d, e). A significant enhancement in cytotoxicity measured by resazurin assay was also observed with T-DM1 doses of 100–300 µg/ml (Fig. 1f). In contrast, pretreatment of 786-0 cells with trastuzumab alone followed by VSVΔ51 infection did not impact microtubule depolymerization or cell death (Fig. 1d, e, g). Importantly, T-DM1 did not lead to similar levels of polynucleation, VSVΔ51 infection, or cell killing in normal GM38 fibroblasts (Fig. 1e, Supplementary Fig. 4) even though they express similar levels of HER2 and bind trastuzumab (Supplementary Figs. 1 and 2). Thus, taken together these data suggest that T-DM1-mediated sensitization to virus infection is cancer-specific and mediated by the MDA payload DM1, as opposed to trastuzumab alone.

We have previously demonstrated that MDAs, in addition to facilitating VSVΔ51 spread by blocking IFNβ secretion, can increase bystander cell killing by virus-induced cytokines such as TNFa. In line with this, we found that T-DM1 increased cell death induced by TNFa in 786-0 cells by ~25%, to a similar degree as colchicine, whereas trastuzumab alone had no impact (Fig. 1h). We also confirmed that pretreatment of 786-0 cells with T-DM1 led to a significant reduction in secreted IFNβ levels 20 h post VSVΔ51 infection while trastuzumab treatment did not (Fig. 1i). To confirm that T-DM1 works by a similar mechanism to naked MDAs, a microarray analysis was conducted on mRNA obtained from cells pretreated with either T-DM1, colchicine, trastuzumab or mock-treated, prior to and following VSVΔ51 infection. Clustering analyses confirmed that the gene expression profile of T-DM1-treated 786-0 cells closely resembled that of colchicine in both infected and uninfected conditions, suggesting again that these drugs work by similar mechanisms (Supplementary Fig. 5a). Indeed, qRT-PCR analysis confirmed downregulation of the interferon stimulated gene known as interferon-induced transmembrane protein (IFITM1) following treatment with T-DM1 or colchicine, with no significant impact of...
trastuzumab alone (Supplementary Fig. 5b). Finally, comparing multi-step and single-step virus growth profiles suggested that in cancer cells, microtubule destabilization induced by T-DM1 has a more significant and direct impact on VSVΔ51 spread rather than replication (Fig. 1j, k), similar to what we observed with colchicine12. Overall, these data demonstrate that, like small molecule MDAs, T-DM1 can increase VSVΔ51 viral spread and oncolysis in VSV-resistant cancer cells by dampening the type I IFN pathway and increasing bystander killing.

**T-DM1 with VSVΔ51 increases oncolysis of HER2+ cancer cells.** 786-0 renal carcinoma cells offer a convenient model to study enhancers of VSVΔ51 given their inherent resistance to this virus. 786-0 cells display detectable HER2 levels as determined by...
HER2 expression was con
tested its effects in 4T1.2 and 4T1.2 stably expressing erbB2/
trastuzumab had no impact on VSV
spread and cell killing. Data reveal a ~2-fold increase in plaque
agarose overlay was added to the wells and cells were
with VSV
zumab, or mock-treated for 3-4 h. This was followed by infection
in 6-well dishes, and pretreated with T-DM1, colchicine, trastu-
mary Fig. 7). To further examine the impact of T-DM1 on
resistance of 786-0 cells to VSV
Delta 51 plaque area ~2× in both 4T1.2 and 4T1.2-
51 is administered 4 h before
DM1 in a murine model of
cancer, where JIMT1 trastuzumab-resistant human breast
cancer, where JIMT1 trastuzumab-resistant human breast
cells, T-DM1 or colchicine increased cell death induced by TNFα
in these cells, with no significant impact of trastuzumab alone
(Fig. 2f). Finally, to determine whether the viral enhancement
observed was dependent on T-DM1 specifically, 786-0 or
JIMT1 cells were pretreated for 2 h with either media alone,
trastuzumab or a suspension of intravenous immunoglobulin
(IVIG) as control, followed by an equivalent concentration of T-
DM1 or media alone for a further 2 h. Cells were then washed and
infected with VSVΔ51 at a low MOI and viral titers quantified by
high-throughput assay. Data reveal that blocking HER2 binding
sites through trastuzumab pretreatment abrogated the increase in
viral titers induced by T-DM1 (Fig. 2g). Taken together, these
data demonstrate that T-DM1 acts through HER2 and can lead to
microtubule disruption and increased VSVΔ51-mediated onco-
lysis in HER2-expressing cancer cells.

T-DM1 with VSVΔ51 improves survival of mice with HER2
+ cancer. It has been previously shown that treatment with microtubule destabilizers in vivo leads to profound vascular
shutdown31. In fact, a number of microtubule stabilizers are being
used as vascular disrupting agents32. However, we have also previously shown that vascular disruption can preclude spread
and delivery of VSVΔ51 throughout the tumor in vivo33. For this
reason, in prior studies we have established a treatment protocol
in tumor-bearing mice, where VSVΔ51 is administered 4 h before
the MDA12. We used a similar approach in order to evaluate the
combination of T-DM1 with VSVΔ51 in a murine model of HER2+
cancer, where JIMT1 trastuzumab-resistant human breast cancer
 xenografts were implanted subcutaneously into nude mice and
allowed to grow to ~100 mm³. Tumor-bearing mice were treated with 1E8 pfu VSVΔ51, or PBS, intratumorally to ensure
virus delivery, followed by 10 mg/kg Kadcyla® (T-DM1), or PBS,
intravenously, 4 h later. This regimen was administered four
times, 7 days apart, and survival monitored over time. The
combination therapy of T-DM1 with VSVΔ51 significantly
improved survival compared to monotherapies (p < 0.0001 com-
pared to PBS; p = 0.039 compared to Kadcyla alone; p = 0.004
compared to VSVΔ51 alone; Gehan–Breslow–Wilcoxon test,
Fig. 3a).
T-DM1 increases VSVΔ51 oncolysis in HER2+ patient samples. Given that the combination of MDAs and VSVΔ51 was previously found to be effective in a murine transgenic model of ovarian cancer, we next examined the relevance of our findings to the treatment of human ovarian cancer. We had access to three patient ascites-derived ovarian cancer cell lines. Upon pretreatment of all three cell lines (AF2028, AF2068 and AF2149) with 100 nM colchicine, there was a robust increase in VSVΔ51-GFP output as measured by fluorescence microscopy (Supplementary Fig. 9a) and viral titer (~10–100-fold, Supplementary Fig. 9b). Western blotting for HER2 (Fig. 3b, Supplementary Fig. 6), flow cytometry (Fig. 3c, Supplementary Fig. 2) or immunofluorescence staining using trastuzumab as a primary antibody (Supplementary Fig. 9c) revealed that only AF2068 overexpressed this...
protein. Treatment of AF2068 with 100 µg/ml T-DM1 resulted in microtubule disruption and evidence of polynucleation as revealed by immunofluorescence staining for tubulin (Fig. 3d). Strikingly, following administration of up to 100 µg/ml of T-DM1, concentrations reflective of recommended clinical dosages, only the HER2-overexpressing AF2068 cells demonstrated a significant ~10-fold boost in VSVΔ51 titer as determined by plaque assay (Fig. 3e) resulting in increased cell death (Supplemental Fig. 9d). Taken together our data demonstrate increased sensitivity of HER2-overexpressing patient-derived cell lines to oncolysis induced by the combination of T-DM1 and VSVΔ51, further supporting the clinical relevance of administering T-DM1 in combination with oncolytic VSV in HER2-overexpressing tumors.

Discussion

The resistance of tumors to oncolytic virotherapy is well recognized as a hurdle to their clinical success11,34. This is why our group and others have spent the last decade devising small molecule-based approaches to sensitize tumors to oncolytic virus infection. This approach, in conjunction with highly attenuated and safe viruses (like VSVΔ51) has the main advantage that it physically separates the oncolytic virus and its enhancing component, giving greater control over infection, ensuring virus safety while promoting anti-cancer efficacy. By the same token, one of the main perceived limitations for this combination approach is toxicity and the potential for off-target effects in normal cells. In an attempt to overcome these limitations, we have harnessed antibody–drug conjugation (ADC) technology as a strategy to enable targeting of these small molecules to HER2+ tumors. Using the clinically approved T-DM1 as proof of concept, we found, that delivering the potent DM1 microtubule destabilizing payload via trastuzumab increases viral spread and bystander killing in HER2+ human cancer cells, analogously to what we have previously shown with “free” small molecule MDAs like colchicine (Figs. 1 and 2). Importantly, T-DM1 did not alter virus growth in normal fibroblasts nor did it lead to substantial bystander killing of those cells (Fig. 1e, Supplementary Fig. 4). Furthermore, DM1-mediated microtubule disruption was dependent on HER2 expression as it was not detected in human HER2-negative murine 4T1.2 cells but was observed in human HER2-overexpressing 4T1.2-HER2 cells, which also demonstrated increased VSVΔ51-mediated oncolysis (Supplementary Fig. 8). Finally, we demonstrate that the combination of VSVΔ51 and T-DM1 significantly improves survival in a human xenograft model of trastuzumab-resistant breast cancer (Fig. 3a) and increases VSVΔ51 growth specifically in HER2-overexpressing ovarian cancer cells derived from ascites obtained from patients (Fig. 3b–e, Supplementary Fig. 9). It is to be noted that while most human cells express detectable levels of HER2, we demonstrate here that HER2-overexpressing cancer cells are more sensitive to lower and clinically relevant concentrations of T-DM1, promoting sensitization to oncolytic VSVΔ51. This further suggests the applicability of this combination regimen towards the treatment of HER2+ cancers more generally, potentially beyond the approved T-DM1 indication in breast cancer.

The repertoire of clinical-stage ADCs continues to expand. While a handful of ADCs have been approved for the treatment of cancer, hundreds of mAbs and ADCs are currently in preclinical studies evaluating a number of different target antigens specific to cancer cells or expressed within the tumor stroma or vasculature20,35. We would expect that conjugation of tubulin inhibitors to other targeted antibodies (e.g. CD30, Adcirca®) would also be effective in increasing the oncolytic efficacy of hradoviruses in these tumor types. While effective with oncolytic hradoviruses like VSV and Maraba, T-DM1 may not be broadly applicable to all oncolytic viruses given MDAs do not similarly enhance the growth of DNA viruses such as vaccinia and HSV-12. Nevertheless, our study provides proof of concept that linking small molecule V5es to targeted antibodies is a viable strategy to synergize with oncolytic hradoviruses such as VSVΔ51.

From our data, it emerges that not only does T-DM1 sensitize HER2+ tumors to oncolytic virus infection, but also that oncolytic VSV provides added benefit in the context of trastuzumab-refractory tumors. This may provide new options for patients that have developed trastuzumab resistance but for which HER2 remains expressed at the surface as is observed in JIMT1. Indeed, the fact that T-DM1 is an approved drug for HER2+ breast cancer and that both VSV and Maraba are undergoing clinical evaluation for solid tumors including breast cancer (NCT02923466, NCT02285816) provides a clear path to clinical translation of this approach.

In short, we demonstrate here for the first time that combining an ADC like T-DM1 with VSVΔ51 can increase viral infection and cytokine-mediated bystander killing of HER2+ tumor cells. Systemic delivery of T-DM1 in combination with oncolytic VSV delayed tumor progression in HER2-overexpressing, but trastuzumab-refractory, JIMT1 human breast cancer xenografts compared to monotherapies, underscoring the potential of this combination in treating both oncolytic virus- and trastuzumab-refractory malignancies. To our knowledge, this is the first proof-of-concept of using an antibody to direct a systematically delivered chemically conjugated viral-sensitizing drug to a tumor, in order to specifically enhance oncolytic virotherapy.

Methods

Cell lines. 786-0 (human renal carcinoma), GM38 (normal human fibroblasts), T47D (human mammary epithelial), MCF7 (human mammary adenocarcinoma), 4T1 (mouse mammary epithelial) and Vero (African Green Monkey kidney,
CCL-81) cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM, HyClone, Waltham, Massachusetts or Corning, Manassas, Virginia) with 10% fetal bovine serum (FBS; VWR, Mississauga, Ontario). SKOV3 (human ovarian) was obtained from ATCC and maintained in Roswell Park Memorial Institute medium (RPMI; ATCC, Cat. # ATCC 30-2001) with 10% FBS. JIMT1 (human herceptin-resistant breast cancer, ACC589) cells were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and cultured in DMEM with 10% FBS. Primary human ovarian cancer cells (AF2028, AF2068, AF2149) were obtained from the Ottawa Ovarian Cancer Tissue Bank under a protocol approved by the Ottawa Health Science Network Research Ethics Board (OHSN-REB 1999540-01H) with informed consent. Cells were isolated from ascites fluid collected from patients with ovarian cancer and maintained in primary culture in RPMI medium supplemented with 10% FBS. 4T1.2 and 4T1.2-HER2 were obtained from the laboratory of Dr. Michael Kershaw and were cultured in RPMI with FBS. All media were supplemented with 1% penicillin–streptomycin solution.
Once tumors were ~100 mm$^3$, mice were treated weekly four times (dotted lines) with the regimen of VSVΔ51-Fluc or PBS (intratumorally) followed by 10 mg/kg T-DM1 (Kadcyla®) or PBS 4 h later (intravenously). Survival was monitored over time ($n = 16–20$ mice per group as indicated, pooled over three independent experiments) and was observed to be significantly reduced with the combination of VSVΔ51 + T-DM1 compared to either monotherapies ($p = 0.039$ compared to Kadcyla; $p = 0.004$ compared to VSV; $p < 0.0001$ compared to PBS, Gehan-Breslow-Wilcoxon test). As Ascal cell lines derived from three different human ovarian cancer patients were each lysed and blotted for HER2 or actin as a loading control. e Mouse breast carcinoma 4T1 cells, and the ascs fluid-derived cell lines were subject to extracellular staining and flow cytometry. Cells were stained with Herceptin® (trastuzumab, 1:1000), followed by goat anti-human IgG-PE (1:100). PE signal (y axis) was analyzed by flow cytometry, median MFI are shown; $n = 3 ± SD.

Fig. 3 T-DM1 increases oncolytic activity of VSVΔ51 in vivo and in human patient samples. a BALB/c nude mice were implanted with $1 \times 10^5$ JIMTI cells. Once tumors were ~100 mm$^3$, mice were treated weekly four times (dotted lines) with the regimen of VSVΔ51-Fluc (1E6 pfu) or PBS (intratumorally) followed by 10 mg/kg T-DM1 (Kadcyla®) or PBS 4 h later (intravenously). Survival was monitored over time ($n = 16–20$ mice per group as indicated, pooled over three independent experiments) and was observed to be significantly reduced with the combination of VSVΔ51 + T-DM1 compared to either monotherapies ($p = 0.039$ compared to Kadcyla; $p = 0.004$ compared to VSV; $p < 0.0001$ compared to PBS, Gehan-Breslow-Wilcoxon test). As Ascal cell lines derived from three different human ovarian cancer patients were each lysed and blotted for HER2 or actin as a loading control. e Mouse breast carcinoma 4T1 cells, and the ascites fluid-derived cell lines were subject to extracellular staining and flow cytometry. Cells were stained with Herceptin® (trastuzumab, 1:1000), followed by goat anti-human IgG-PE (1:100). PE signal (y axis) was analyzed by flow cytometry, median MFI are shown; $n = 3 ± SD.

Cell viability assay. The metabolic activity of the cells was assessed using alamarBlue (BioRad, Mississauga, Canada) or resazurin sodium salt (Sigma-Aldrich) according to the manufacturer’s protocol. Treated and/or infected cells were administered 10% (volume/volume) final, resazurin in each well and incubated for 2–4 h, depending on the cell line. Fluorescence was measured at 590 nm upon excitation at 530 nm using a Fluoroskan Ascent FL (Thermo Scientific, Beverly, MA) or the BioTek Microplate Reader (BioTek, Winooski, VT, USA).

IFNγ ELISA. 786-0 cells were plated to confluence in 12-well plates and infected overnight 37 °C in a 5% CO2 humidified incubator. Cells were then treated with specified drug concentrations and infected 4 h later with VSVΔ51-GFP at an MOI of 0.1 or mock infected. Twenty hours post infection, cell supernatant was collected and frozen at −80 °C. Supernatants were then thawed and plated at 3000 cells per well. Viral titres contained within the cleared supernatant was subsequently subject to 0.22 µm membrane filtration and purified using 5–50% Optiprep (Sigma-Aldrich, Oakville, ON, Canada, Cat. # D1956) gradient36. The purified virus suspension was aliquoted and frozen at −80 °C. For all virus infections, viruses were diluted in serum-free DMEM to obtain the specified MOI, or for mock infection cells were supplemented with an equal volume of serum-free DMEM. For high-throughput luciferase titration29, Vero cells were prepared to be 95–100% confluent in opaque white 96-well plates in 100 µl complete DMEM supplemented with 30 nM HEPES. VSVΔ51-Fluc infected samples to be titered were transferred (25 µl/well) onto the Vero cells in 96-well plates. Standard curve prepared from a purified virus stock of known titer and diluted from 10$^{-6}$–1 PFU/mL in duplicate for each 96-well plate. Vero plates were then incubated for 5 h at 37 °C 5% CO2, following which aD-luciferin solution was automatically dispensed at 25 µl per well. Luminescence was read at an appropriate time after 2 h of incubation. Cells were then treated with trastuzumab or IVIG (786-0 cells) and frozen with 1:100 protease inhibitor complex (Roche, Cat. # 11697498001). Verikine human IFNγ ELISA (PBL Interferon Source, Piscataway, NJ, USA, Cat. # 41410) kits were used following the manufacturer’s instructions and IFNγ values (pg/ml) were interpolated from the obtained standard curve.

Cell lysis and western blotting. Whole-cell lysates were obtained by lysing the cells in 30 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM Na$_3$VO$_4$, 10 mM EDTA, 100 mM NaF, 10 mM Na$_3$PO$_4$, protease inhibitor cocktail (Roche) and 1% Triton X-100 on ice. Protein concentration was determined by Bradford assay (BioRad, Cat. # 5000002) and 20–100 µg of cell extract were run using the NuPage SDS-PAGE system (Invitrogen, Carlsbad, CA, USA, Cat. # NP0322) and transferred onto a nitrocellulose membrane (BioRad, Cat. # 1620115). Membranes were blocked with 5% milk in TBS-T for 1 h followed by a secondary donkey anti-goat antibody conjugated to Alexa 594 (Invitrogen, Cat. # A-11058) or goat anti-mouse antibody conjugated to Alexa 488 (Invitrogen, Cat. # A-11005) or goat anti-rabbit antibody conjugated to Alexa 647 (Invitrogen, Cat. # A-11007). Membranes were washed with TBS-T and then incubated with a goat anti α- tubulin primary antibody (1:200, Abcam, Cat. # AB6046) or mouse anti-a- tubulin (1:50, Santa Cruz Biotechnology, Dallas, TX, USA, Cat. # SC-8035), in 1% bovine serum albumin (BSA)-PBS$. Following washes, coverslips were incubated for 1 h at room temperature with a secondary donkey anti-goat antibody conjugated to Alexa 594 (Invitrogen, Cat. # A-11058) or goat anti-mouse antibody conjugated to Alexa 488 (Invitrogen, Cat. # A-11001) diluted 1:200 in 1% BSA. Coverslips were mounted on slides with ProLong Gold Antifade reagent with DAPI (Life technologies/Thermo Scientific, Burlington, Ontario, Cat. # P36930) and stored at 4 °C. Images were taken using the AxioCam HRm camera (Carl Zeiss Ltd, Toronto ON) mounted on the Zeiss Axioscope Imager. Virus and infected cells were manually counted from 6 fields per experimental condition, in triplicate at 40× with a minimum of 100 cells counted per condition.

Immunofluorescence staining. Cells were plated on sterile glass coverslips in 12-well plates and incubated overnight 37 °C in a 5% CO2 humidified incubator. Cells were then treated at the specified drug concentrations for 4 h and infected with VSVΔ51-GFP at an MOI of 0.1 or mock infected. Twenty-four hours post infection, cells were fixed with 4% paraformaldehyde, quenched with 100 mM glycine in PBS$^*$ (supplemented with 1 mM CaCl$_2$ and 0.5 mM MgCl$_2$), permeabilized with 0.1% Triton-X 100, then blocked in 5% BSA in PBS$^*$. Slides were subsequently incubated overnight at 4 °C in a humidified chamber with a goat anti β-tubulin primary antibody (1:200, Abcam, Cat. # AB6046) or mouse anti-a- tubulin (1:50, Santa Cruz Biotechnology, Dallas, TX, USA, Cat. # SC-8035), in 1% bovine serum albumin (BSA)-PBS$. Following washes, coverslips were incubated for 1 h at room temperature with a secondary donkey anti-goat antibody conjugated to Alexa 594 (Invitrogen, Cat. # A-11058) or goat anti-mouse antibody conjugated to Alexa 488 (Invitrogen, Cat. # A-11001) diluted 1:200 in 1% BSA. Coverslips were mounted on slides with ProLong Gold Antifade reagent with DAPI (Life technologies/Thermo Scientific, Burlington, Ontario, Cat. # P36930) and stored at 4 °C. Images were taken using the AxioCam HRm camera (Carl Zeiss Ltd, Toronto ON) mounted on the Zeiss Axioscope Imager. Virus and infected cells were manually counted from 6 fields per experimental condition, in triplicate at 40× with a minimum of 100 cells counted per condition.

Flow cytometry. 1 × 10$^6$ cells were resuspended in 200 µl of FACS buffer (0.5% BSA-PBS$^*$) and transferred to round-bottom 96-well plates and stained with Fixable Viability Dye 510 (BD Horizon, San Jose, California, USA, Cat. # 564068) at 1:1000 for 30 min at 4 °C in the dark. Cells were then pelleted at 1500 rpm, 5 min, at 4 °C. Washed with FACS buffer, then stained with trastuzumab at 1:1000 in FACS buffer (final concentration 21 µg/ml) for 60 min at 4 °C in the dark. Cells were then
pelleted, washed, and stained with goat anti-human IgG-Fc (Invitrogen, Cat. # PA1-80978) at 1:100, for 60 min at 4 °C in the dark. Cells were then washed, pelleted, and resuspended in 1% PFA-PBS and stored overnight at 4 °C. Immediately prior to acquisition, samples were filtered (BioDesign Inc., Carmel, New York, USA, 500 µl MicroSieves, 50 µm pore size) then subjected to flow cytometry using a BD LSRFortessa Flow Cytometer (BD Horizons). Data were analyzed using FlowJo v10.6 software. Unstained controls were prepared in parallel, and PE-treated beads were used for compensation and gating.

**JIMT1 xenograft model.** Six- to eight-week-old female CD1 nude mice (Crl:CD1-Foxn1nu) purchased from Charles River Laboratories, Wilmington, Massachusetts, USA, Strain code # 086) were implanted subcutaneously with 1×10^6 JIMT1 cells resuspended in 100 µl PBS and 100 µl GelTrax (ThermoFisher, Cat. # A1143201), for a 200 µl total volume. When tumors reached approximately 100 mm³, mice were treated with the indicated treatments. Tumor volume was assessed using D ²πl Grabber software (BioDesign Inc., Carmel, New York, USA) every other day using an electronic caliper and volumes were calculated as (length x width²)/2. Tumor data were assessed using D'Agostino & Pearson omnibus and Shapiro-Wilk normality tests. Alpha levels for all tests were 0.05 (conventional replicates are indicated by a number in parentheses). Two-way ANOVA with Sidak or Dunnett's post hoc test was used when groups were split on two independent variables. Normal distribution of the data was assessed using D'Agostino & Pearson omnibus and Shapiro–Wilk normality tests. Alpha levels for all tests were 0.05 (confidence levels of 95%). Biologically relevant data are available from the authors upon request to the corresponding author.
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Author contributions

R.A., V.G. and J.-S.D. participated in the conception and design of the studies. R.A., Z.T., V.G., M.S., K.S. and O.V. performed in vitro experiments. A.C., M.S., R.A., V.G., A.B. and Z.T. performed and assisted with animal experiments. Z.T., A.J., S.B., N.A., A.L. and F.T. performed flow cytometry acquisition and analysis. E.M. and B.V. established and provided patient-derived cell lines. R.A., V.G., Z.T. and J.-S.D. performed the statistical analysis and contributed to the manuscript. A.C., M.S., R.A., V.G. and J.-S.D. participated in the conception and design of the studies. R.A., Z.T., A.J., S.B., N.A., A.L. and F.T. performed flow cytometry acquisition and analysis. E.M. and B.V. established and provided patient-derived cell lines. R.A., V.G., Z.T. and J.-S.D. drafted the manuscript with editorial contributions from M.S., F.T. and B.V.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.-S.D.

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