Gene Therapy (2008) 15, 753–758

www.nature.com/gt

REVIEW

Integrating the biological characteristics of oncolytic viruses and immune cells can optimize therapeutic benefits of cell-based delivery

SH Thorne[1] and CH Contag[2]
[1]University of Pittsburgh Cancer Institute and Department of Surgical Oncology, University of Pittsburgh, Pittsburgh, PA, USA and [2]Department of Pediatrics, Department of Radiology and Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA

Despite significant advances in the development of tumor-selective agents, strategies for effective delivery of these agents across biological barriers to cells within the tumor microenvironment has been limiting. One tactical approach to overcoming biological barriers is to use cells as delivery vehicles, and a variety of different cell types have been investigated with a range of agents. In addition to transporting agents with targeted delivery, cells can also produce their own tumoricidal effect, conceal a payload from an immune response, amplify a selective agent at the target site and facilitate an antitumor immune response. We have reported a therapeutic combination consisting of cytokine induced killer cells and an oncolytic vaccinia virus with many of these features that led to therapeutic synergy in animal models of human cancer. The synergy was due to the interaction of the two agents to enhance the antitumor benefits of each individual component. As both of these agents display broad tumor-targeting potential and possess unique tumor killing mechanisms, together they were able to recognize and destroy a far greater number of malignant cells within the heterogeneous tumor than either agent alone. Effective cancer therapy will require recognition and elimination of the root of the disease, the cancer stem cell, and the combination of CIK cells and oncolytic vaccinia viruses has this potential. To create effective tumor-selective agents the viruses are modified to take advantage of the unique biology of the cancer cell. Similarly, if we are to develop targeted therapies that are sufficiently multifaceted to eliminate cancer cells at all stages of disease, we should integrate the virus into the unique biology of the cell delivery vehicle.
Gene Therapy (2008) **15,** 753–758; doi:10.1038/gt.2008.42; published online 20 March 2008

Keywords: *oncolytic; vaccinia virus; immunotherapy*

Similar to chemotherapies, development of biological therapies, such as oncolytic viruses, is based on targeting the unique biology of cancer cells to achieve an effective therapeutic index against malignant versus normal cells. By necessity, measurement of therapeutic indices are performed in cell culture. The delivery tools that effectively transport agents to the tumor target in living subjects are absent from these stages of development. It is not surprising, therefore, that despite the development of extremely potent anticancer drugs and biological therapies, their designs lack mechanisms for crossing biological barriers and for directed delivery to malignant cells *in vivo*. Selective delivery of a therapeutic agent to a target cell can enhance the therapeutic index *in vivo* due to selective accumulation of the agent within the tumor. This can both effectively reduce exposure of normal tissues to toxic agents and achieve a higher effective concentration at the tumor site.

Delivery strategies can be passive and depend on leaky vasculature and an increased blood pool, or they can incorporate active targeting of specific aspects of malignant cells and/or the microenvironment of the tumor. The objective is that systemically delivered therapies accumulate at the tumor site. Active delivery can be based on molecular recognition of tumor markers or antigens that are accessible to blood flow (for example, peptides and antibodies directed against tumor-specific endothelial cells). In the case of oncolytic viruses, these molecules can be used to alter the tropism of viral agents such that they recognize receptors expressed on tumor cells or tumor endothelium.[1–5] Similarly nanoparticles that are coated with these domains or ligands accumulate in the tumor.[6,7] Blending an active targeting mechanism with a biologically active delivery vehicle, for example an immune cell, could increase the beneficial effect. Such approaches rely on cell types that are recruited by, or naturally traffic to, the tumor microenvironment, and these act as carrier vehicles to deliver a therapeutic agent or payload to the tumor.[8,9] Tumor-targeting cells that have been used for such directed therapy include hematopoietic or immune cells, or precursor or stem cells. As these cells have their own biology that can be modified and exploited for improved therapies, understanding and utilizing the biology of the vehicle will be essential for optimizing these approaches.

Correspondence: Dr SH Thorne, Department of Surgical Oncology, University of Pittsburgh, 5115 Centre Avenue, Pittsburgh, PA 15232, USA.
E-mail: thornesh@upmc.edu
Received 14 February 2008; accepted 16 February 2008; published online 20 March 2008

Among the variety of strategies for utilizing cell-based carriers, the most basic approach uses cell types with no intrinsic tumor trafficking ability. These cells are used to either express a transgene within a tumor, or as a concealment mechanism to improve delivery of a viral (or similarly antigenic) agent, in attempts to circumvent an antiviral immune response (usually incorporating a passive delivery approach). Cells can also improve microdistribution after direct delivery to a tumor by moving within the tumor environment away from the initial sites of injection, so giving the therapy an improved biodistribution.[10] In a more complex approach, cells that are known to traffic to tumors are used as targeting vehicles to actively carry a viral agent or a transgene to the tumor, in essence hijacking the trafficking ability of the cell. In some examples of this approach, the cell itself has therapeutic potential resulting in additive antitumor effects between the carrier and the passenger.[11] Finally, in the most complex scenario, a tumor-targeting, cell-based therapeutic will be used as a carrier vehicle in conjunction with a therapeutic passenger. The combination may have additive effects or the biology of the targeting system and the payload can be integrated leading to synergistic tumor-killing.

Oncolytic agents based on a wide range of viruses have been developed and tested as cancer therapies (for review see SH Thorne *et al*.[12] and KA Parato *et al*.[13]). Among the different viruses that have been studied, vaccinia virus strains have a number of characteristics that contribute to their enhanced potential as oncolytic agents; vaccinia viruses demonstrate rapid spread, potent cytolytic ability, a broad range of host cell tropism, a large capacity for genetic payload and a high degree of specificity for tumor targets produced through selective deletion of viral genes.[14,15] These agents also display some degree of systemic delivery potential to the tumor, primarily due to their evolved ability to travel relatively undetected within the blood. However, because they possess the ability to infect a broad range of cell types, only a small amount of virus delivered intravenously will infect the tumor, with the majority undergoing non-permissive or abortive infections of non-tumor tissue. Therefore, despite the promising results of vaccina virus-based cancer therapies,[14–18] it appears that for effective treatment of cancers it will be necessary to both improve methods of delivery and use of vaccinia in therapeutic combinations.

Given the complexity of combined biological therapies and the requirement that delivery methods be evaluated in living animals, effective development of these approaches requires methods of study that refine animal models of cancer and accelerate their analyses. The tools being developed in the field of molecular imaging have addressed this previously unmet need and have enhanced the discovery and development of novel therapies for cancer.[19,20] These imaging methods utilize molecular probes to label the cancer cell, the delivery vehicle and/or the therapeutic agent such that their numbers and location can be determined in the animal over time.[21] Such imaging tools offer the power of repeated measures without killing the animal yielding improved data while offering a significant savings in the numbers of animals required and cost of the study.[20,22,23] Among the molecular imaging methods described to date, *in vivo* bioluminescence imaging [19,22] has emerged as one of the most versatile and sensitive methods for *in vivo* studies of biology and therapeutic responses in animal models. As such bioluminescence imaging has been used to advance our understanding of a number of chemotherapeutic agents[24,25] and biological therapies[26,27] for cancer and other diseases.[28]

The combination of cytokine induced killer (CIK) cells as the carrier vehicle and oncolytic vaccinia virus as its biological payload fall into the category of cell-based combination therapies that may offer additive or synergistic therapeutic benefits.[21] CIK cells have been shown to display impressive tumor-trafficking potential following systemic delivery in pre-clinical models,[29] with the majority of the cells detected in the tumor by 72 h after intravenous delivery (Figure 1). These cells were developed as a tumorical population of immune cells that have characteristics of both T cells and natural killer (NK) cells.[31] Although these cells display cytolytic ability in correlative cell culture assays and *in vivo* within the tumor micro-environment, large numbers of CIK cells are typically required to achieve efficient tumor clearance—effector to target ratios of 10 or 20 to 1.[30] Oncolytic vaccinia viruses have been developed as highly lytic agents with good tumor selectivity, but they are limited in their biodistribution.

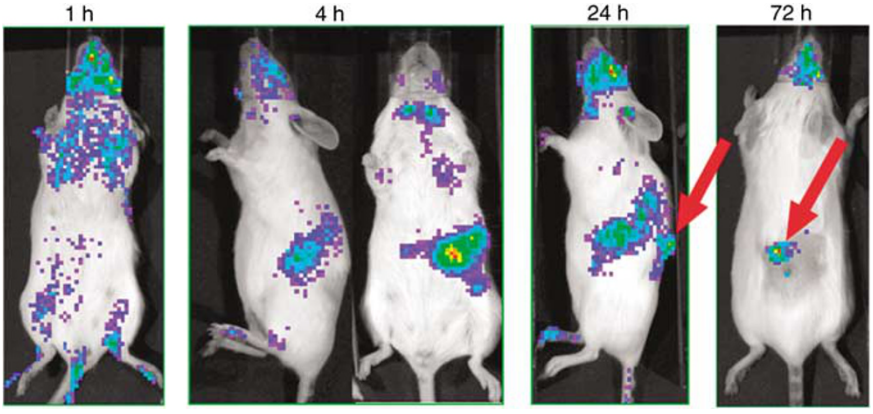

Figure 1 Mouse splenocytes were expanded and treated with cytokines, as described,[30] for enrichment of cytokine induced killer (CIK) cells. These cells were labeled by retroviral transduction using a retroviral vector containing the luciferase gene (pGL4 from Promega Corp, Madison, WI, USA) and delivered intravenously to a mouse (BALB/c) carrying a subcutaneous mammary carcinoma cell line (4T1; arrows) implanted on the back of the animal 10 days earlier. Subsequent trafficking patterns of the CIK cells were followed by bioluminescence imaging (BLI) following delivery of luciferin, using an IVIS100 system (Xenogen Products from Caliper Life Sciences, Alameda, CA, USA).

Both CIK cells and oncolytic vaccinia virus strains have displayed encouraging results in Phase I clinical testing;[17,32] however, the described limitations of each therapy suggested that the combination may offer complementary effects that would enhance the overall therapeutic outcome. We initially proposed that by utilizing CIK cells as a carrier vehicle to deliver oncolytic vaccinia to the tumor we may be able to overcome the deficiencies of each therapy as a single agent (inefficient tumor delivery of vaccinia strains and limited tumor cell killing of CIK cells), while retaining their benefits. To investigate this possibility it was necessary to determine if the tumor-selective vaccinia viruses would replicate in CIK cells and if so would the virus interfere with the trafficking and tumoricidal properties of the CIK cells.

We observed that vaccinia strains are capable of infecting CIK cells, but replicate with unusual kinetics, demonstrating a prolonged eclipse period (48 h) during which the virus lies dormant within the cells.[21] During this time, we were unable to detect changes in the cytolytic behavior, cell surface marker expression, cytokine production or tumor trafficking potential of the CIK cells. Essentially, the function of the CIK cells is unaffected by the viral infection during this eclipse period, allowing efficient delivery of the virus directly to the tumor (even intraperitoneal tumors could be targeted following intravenous delivery of the agent[21]). Furthermore, the CIK cells do not display viral antigens at the cell surface during this eclipse phase, meaning that delivery can be achieved even in the face of an antiviral immune response. After the eclipse phase, however, the virus performs a limited replication, resulting in lysis of the infected CIK cells and release of multiple infectious viral particles into the tumor microenvironment leading to an amplification of the therapeutic agent within the tumor—one aspect of therapeutic synergy.

The fact that the majority of CIK cells delivered into the blood stream end up within the tumor by 48–72 h after treatment means that a much greater amount of the viral dose will ultimately be released directly within the tumor compared to intravenous delivery of the oncolytic vaccinia as a single agent. This results in greater on-target delivery and less off-target toxicities improving the tumor selectivity. The subsequent rapid replication and spread of the virus within the tumor results in even small amounts of virus initially delivered to the tumor, producing a dramatic antitumor effect.

For many oncolytic viruses the replication, and not infection *per se*, is tumor selective. Therefore, when oncolytic viruses are delivered as a single agent through the blood stream, they will initially infect the first cells they come in contact with. Even with more selective and targeted viruses, they will only be able to infect the first cells within the tumor that they come into contact with (that is, cells close to or exposed to the tumor vasculature). Although subsequent spread into the bulk of the tumor can then be achieved, viral infection is normally prematurely compromised by the inability of the virus to spread beyond physical barriers within the complex tumor microenvironment, such as areas of necrotic tissue, or areas of extracellular matrix. Viral spread may be further contained by an antiviral immune response.

Limited infections are demonstrated by histology[21] and by viral gene expression (as measured by bioluminescence imaging) that correlates closely with overall numbers of viral particles within the tumor. Since optical imaging is surface weighted, as the virus moves through susceptible tissues infecting surrounding cancer cells the signal from viral reporter gene expression (bioluminescence) drops off relative to numbers of particles (Figure 2). This discordance may also represent compartmentalization of virus in acellular regions of the tumor, that is, entrapment of virus within necrotic pockets of destroyed tumor tissue, physically separated from the remainder of the tumor by extracellular matrix. This highlights the importance of being able to biodistribute the virus efficiently throughout the entire tumor tissue to obtain maximum benefits. A number of groups have described means of increasing the therapeutic benefit of oncolytic viruses through combination with collagenase or through expression of genes able to degrade the extracellular matrix;[33,34] however, this still fails to overcome the inability of viruses to actively traffic to or within tumors. It appears that the use of cells capable of actively extravascating from the blood supply and moving within the tumor microenvironment, so delivering their viral passengers at locations throughout the tumor, results in even greater viral penetration within the tumor and increased therapeutic benefit.

Delivery of oncolytic viruses as single agents through the blood stream (where possible), leads to an initial infection of cells in or around the tumor endothelium, and this has been found to lead to subsequent vascular collapse, cutting the blood supply to the tumor.[35,36] This suggests that some cell-free virus would be beneficial. It is likely that the CIK-vaccinia dual biotherapy will also achieve this effect due to the delivery of free virus in conjunction with infected and uninfected CIK cells. Under the protocol that we used, we found that at the time of inoculation the combination therapy contains about 20% infected and 80% uninfected CIK cells, and also significant amounts of free, or cell-associated virus. Thus, some CIK cells will remain within the tumor as

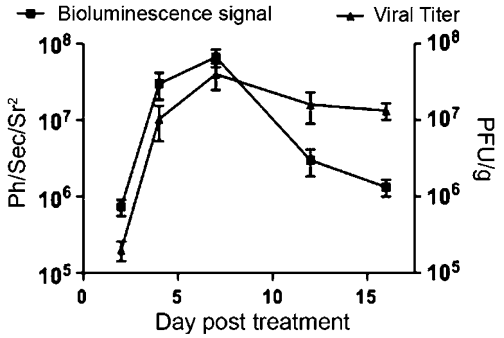

Figure 2 Mice (BALB/c) bearing subcutaneous 4T1 tumors as in Figure 1 were treated (day 0) via a single tail vein injection of 1×10^7 PFU of vaccinia strain Western Reserve carrying a mutation in the thymidine kinase gene and expressing luciferase from the pSE/L promoter. Animals ($n = 4$) were imaged at times after treatment as before, and the bioluminescence signal produced within the tumor at different times was determined by drawing a region of interest over the tumor and measuring signal (using Living Image software, Caliper Life Science). Additional mice were killed at each time point and their tumors extracted, weighed and the tissues homogenized. The number of infectious viral units per gram of tumor was determined by plaque assay ($n = 3$ per time point).

tumoricidal cells many days after initial treatment (that is, not destroyed by viral infection), and that some virus will be likely to reach the tumor target as a single agent and affect the endothelium. It is unlikely that significant numbers of uninfected CIK cells will become infected within the tumor as tumor cells are dramatically more susceptible to oncolytic viral replication than the CIK cells.

This mixed therapeutic effect is an important factor that appears to help further enhance both the therapeutic benefits and the complex and beneficial interactions between the immune cell and the virus. With human tumor cell lines, it has been shown that some are naturally resistant to CIK cell-mediated killing. CIK cells recognize their tumor targets through the interaction between the CIK cell surface-expressed NKG2D receptor and tumor cell-surface expressed NKG2D ligands.[37] NKG2D ligands, such as MICA and MICB in humans, are stress response ligands that are commonly upregulated in tumor tissues due to the multitude of stresses that are encountered within this environment (for example, hypoxic or pH stress, limited nutrients or increased build up of waste products), making tumor cells natural targets of NK or NK-T cells.[38,39] Although about 80% of human tumor cells are recognized by the NKG2D receptor, some tumors develop means to counter this by downregulating NKG2D ligand expression on the cell surface. However, viral infection is also a stress condition and infection has been shown to increase NKG2D ligand cell surface expression.[40] Thus, the oncolytic virus may enhance the activity of CIK cells. We have demonstrated that vaccinia infection of at least some tumor cells results in increased MICA or MICB expression on the surface of the cell.[21] As a result it appears that free virus, delivered as part of the initial inoculum, is capable of infecting tumors that are naturally resistant to CIK cell-mediated killing. This infection results in upregulation of MICA and MICB expression on the surface of the tumor cells, so making the previously resistant cells susceptible to recognition by CIK cells. This has the consequence of increasing the numbers of CIK cells infiltrating the tumor, so increasing the delivery of oncolytic vaccinia, which further increases MICA and MICB expression. In this way a positive feedback loop can be created leading to increased synergy.

We have also observed additional mechanisms of synergy that involve immune activation. We demonstrated that the combination of immunostimulatory molecules released by the activated CIK cells and the viral infection within the tumor releasing viral and tumor antigens, as the tumor cells are lysed, results in an increased activation of the host immune response relative to either therapy alone (Thorne and Contag, unpublished data). This leads to the creation of significant numbers of CD4 and CD8 positive cells that are found to be activated by relevant tumor antigens *ex vivo*. It appears that the combination of immune cell and viral infection within the tumor has the potential to vaccinate the host *in situ*, so priming the host's immune system to help clear residual tumor cells, and create an immune memory response that can help maintain the long-term disease-free state that is observed in our studies.

Combination therapies may benefit from broad and overlapping tumor recognition mechanisms as tumors are heterogeneous with end stage disease often differing from the initial transformed cells that may comprise the 'root' or foundation of disease. For this reason using T-cell antigen recognition or monoclonal antibodies to redirect therapeutic agents may be limited. That is, since these approaches rely on the recognition of a single cell surface receptor to distinguish target from non-target cells, and the receptor may be expressed on only a subset of tumor cells, or else it may easily be downregulated under conditions of negative selection, there may be cells that escape such narrowly directed therapies. Alternatively, even widespread tumor targets may not be accessible given the endothelial barrier of the tumor vasculature, and so cannot be detected by intravenously delivered therapies.

The CIK-vaccinia dual biotherapy, however, has the potential to recognize a wide variety of cell surface markers on tumor cells and on tumor endothelial cells to distinguish tumor and non-tumor tissues. The NKG2D receptor on CIK cells recognizes a family of about 12 NKG2D ligands on tumor targets, and uses several adhesion molecules to recognize the abnormal tumor vasculature. The viral component of the dual biotherapy is able to infect almost all known cell types, and so is not limited by the expression of cell surface receptors. This means that even if only a small subset of cells within the tumor express an NKG2D ligand and are recognized by the CIK cells, the subsequent release of infectious virus from the CIK cell infiltrates within the tumor may result in therapeutic targeting of the remaining tumor cells.

Many cancer therapies rely on targeting the products of a single genetic lesion, often members of a signaling pathway within the tumor cell (for example, tyrosine kinase inhibitors) or rely on a single mechanism of tumor cell killing to destroy tumor cells (most chemotherapeutics rely on apoptosis as a killing mechanism). The selective pressures of such therapies may lead to the creation of alternative or redundant cell-signaling defects or the loss of apoptotic potential, leading to the outgrowth and escape of resistant tumor cells. The use of combination therapies is a proven strategy in chemotherapy, and similarly in biotherapies a simultaneous assault on a variety of tumor markers will produce a greater therapeutic effect and perhaps eliminate a sufficient number of tumor cells to enable immune clearance of the remaining malignant cells.

In addition to the effects of selective pressure due to directed therapies, tumors are naturally a heterogeneous mix of cancer cells that display a range of different properties such as different proliferation potential and proliferation states that are likely due to the physiological heterogeneity of the tumor microenvironment. The emerging concept of the cancer stem cell, that there is a cell type ultimately responsible for regenerating the tumor following a treatment, and for tumor metastasis, suggests that these cells are phenotypically distinct from the rest of the tumor.[41–43] To successfully destroy the entire tumor, and any residual disease that serves as a source of relapse, it will be imperative that such therapies are developed which recognize and kill all tumor cell subtypes, including the cancer stem cell. To achieve this it is likely that multi-component, broadly active combination therapies will be needed.

Although no dual biotherapy consisting of a carrier cell and oncolytic virus passenger has yet been tested in

the clinic, the fact that both CIK cells and oncolytic vaccinia strains have undergone clinical testing with positive results and little or no associated toxicities, coupled with the highly encouraging pre-clinical data using this dual biotherapy make this a strong candidate for rapid clinical development.

The CIK-vaccinia dual biotherapy already relies on a complex set of interactions between the CIK cell, the oncolytic virus, the tumor and the host's immune response; however, in future developments a further component or components may be added. For example, either the CIK cells or virus could be engineered to express therapeutic transgenes to further enhance tumor cell killing. As with the rational selection of a suitable carrier cell and viral agent though, an understanding of how a transgene will interact with the biology of the host, of the other components of the therapy and of the cellular target will be vital to optimize the overall tumor killing potential, and to prevent the risk of antagonistic effects occurring between the different therapeutic agents that would reduce the overall benefits. In this way the therapeutic approach would be structured like a 'Russian Doll' with multiple concentric layers of therapeutic agents involved in consecutive steps in the destruction of the tumor target.

However, with extra levels of complexity comes an additional onus on exerting tight control over the components of the system, both to improve efficacy and to ensure absolute safety. For example, it is possible that expression of a therapeutic transgene from a viral payload, such as a vaccinia strain, may disrupt the tumor-targeting potential of the CIK or related cell carrier or else premature killing of the infected carrier cell may result in limited ability for the viral payload to replicate. For this reason, it would be beneficial to control the transgene expression levels and timing. This might be achieved by the use of tissue or tumor specific promoters driving the transgene (so limiting transgene expression to cells within the tumor),[44] or else, the use of inducible or repressible systems to control viral gene expression may be incorporated.[45] These have an additional advantage that transgene expression may subsequently be switched off if necessary to prevent adverse events.

It, therefore, seems likely that the use of immune cell carrier vehicles such as CIK cells to deliver oncolytic virus strains to tumors has the potential to become a major weapon in the fight against cancer. However, this potential cannot be realized until we see clinical safety and efficacy data with such a system. The CIK-vaccinia dual biotherapy has the potential for rapid clinical development and is an area of active investigation.

References

1 Akiyama M, Thorne S, Kirn D, Roelvink PW, Einfeld DA, King CR *et al.* Ablating CAR and integrin binding in adenovirus vectors reduces nontarget organ transduction and permits sustained bloodstream persistence following intraperitoneal administration. *Mol Ther* 2004; **9**: 218–230.
2 Wickham TJ. Ligand-directed targeting of genes to the site of disease. *Nat Med* 2003; **9**: 135–139.
3 Douglas JT, Rogers BE, Rosenfeld ME, Michael SI, Feng M, Curiel DT. Targeted gene delivery by tropism-modified adenoviral vectors. *Nat Biotechnol* 1996; **14**: 1574–1578.
4 Nakamura T, Peng KW, Harvey M, Greiner S, Lorimer IA, James CD *et al.* Rescue and propagation of fully retargeted oncolytic measles viruses. *Nat Biotechnol* 2005; **23**: 209–214.
5 Nakamura T, Peng KW, Vongpunsawad S, Harvey M, Mizuguchi H, Hayakawa T *et al.* Antibody-targeted cell fusion. *Nat Biotechnol* 2004; **22**: 331–336.
6 Gao X, Cui Y, Levenson RM, Chung LW, Nie S. *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat Biotechnol* 2004; **22**: 969–976.
7 Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld RA, Xiang R *et al.* Tumor regression by targeted gene delivery to the neovasculature. *Science* 2002; **296**: 2404–2407.
8 Thorne SH. Strategies to achieve systemic delivery of therapeutic cells and microbes to tumors. *Expert Opin Biol Ther* 2007; **7**: 41–51.
9 Thorne SH, Contag CH. Combining immune cell and viral therapy for the treatment of cancer. *Cell Mol Life Sci* 2007; **64**: 1449–1451.
10 Power AT, Wang J, Falls TJ, Paterson JM, Parato KA, Lichty BD *et al.* Carrier cell-based delivery of an oncolytic virus circumvents antiviral immunity. *Mol Ther* 2007; **15**: 123–130.
11 Cole C, Qiao J, Kottke T, Diaz RM, Ahmed A, Sanchez-Perez L *et al.* Tumor-targeted, systemic delivery of therapeutic viral vectors using hitchhiking on antigen-specific T cells. *Nat Med* 2005; **11**: 1073–1081.
12 Thorne SH, Hermiston T, Kirn D. Oncolytic virotherapy: approaches to tumor targeting and enhancing antitumor effects. *Semin Oncol* 2005; **32**: 537–548.
13 Parato KA, Senger D, Forsyth PA, Bell JC. Recent progress in the battle between oncolytic viruses and tumours. *Nat Rev Cancer* 2005; **5**: 965–976.
14 Thorne SH, Hwang TH, O'Gorman WE, Bartlett DL, Sei S, Kanji F *et al.* Rational strain selection and engineering creates a broad-spectrum, systemically effective oncolytic poxvirus, JX-963. *J Clin Invest* 2007; **117**: 3350–3358.
15 McCart JA, Ward JM, Lee J, Hu Y, Alexander HR, Libutti SK *et al.* Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. *Cancer Res* 2001; **61**: 8751–8757.
16 Zhang Q, Yu YA, Wang E, Chen N, Danner RL, Munson PJ *et al.* Eradication of solid human breast tumors in nude mice with an intravenously injected light-emitting oncolytic vaccinia virus. *Cancer Res* 2007; **67**: 10038–10046.
17 Mastrangelo MJ, Maguire Jr HC, Eisenlohr LC, Laughlin CE, Monken CE, McCue PA *et al.* Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. *Cancer Gene Ther* 1999; **6**: 409–422.
18 Kirn DH, Wang Y, Le Boeuf F, Bell J, Thorne SH. Targeting of interferon-beta to produce a specific, multi-mechanistic oncolytic vaccinia virus. *PLoS Med* 2007; **4**: e353.
19 Thorne SH, Contag CH. Using *in vivo* bioluminescence imaging to shed light on cancer biology. *Proc IEEE* 2005; **93**: 750–762.
20 Ntziachristos V, Ripoll J, Wang LV, Weissleder R. Looking and listening to light: the evolution of whole-body photonic imaging. *Nat Biotechnol* 2005; **23**: 313–320.
21 Thorne SH, Negrin RS, Contag CH. Synergistic antitumor effects of immune cell-viral biotherapy. *Science* 2006; **311**: 1780–1784.
22 Contag CH, Ross BD. It's not just about anatomy: *in vivo* bioluminescence imaging as an eyepiece into biology. *J Magn Reson Imaging* 2002; **16**: 378–387.
23 Gambhir SS. Molecular imaging of cancer with positron emission tomography. *Nat Rev Cancer* 2002; **2**: 683–693.
24 Mandl SJ, Mari C, Edinger M, Negrin RS, Tait JF, Contag CH *et al.* Multi-modality imaging identifies key times for annexin V imaging as an early predictor of therapeutic outcome. *Mol Imaging* 2004; **3**: 1–8.
25 Rehemtulla A, Stegman LD, Cardozo SJ, Gupta S, Hall DE, Contag CH *et al.* Rapid and quantitative assessment of cancer

treatment response using *in vivo* bioluminescence imaging. *Neoplasia* 2000; **2**: 491–495.
26 Yu YA, Shabahang S, Timiryasova TM, Zhang Q, Beltz R, Gentschev I *et al*. Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. *Nat Biotechnol* 2004; **22**: 313–320.
27 De A, Loening AM, Gambhir SS. An improved bioluminescence resonance energy transfer strategy for imaging intracellular events in single cells and living subjects. *Cancer Res* 2007; **67**: 7175–7183.
28 Contag CH, Bachmann MH. Advances in *in vivo* bioluminescence imaging of gene expression. *Annu Rev Biomed Eng* 2002; **4**: 235–260.
29 Edinger M, Cao YA, Verneris MR, Bachmann MH, Contag CH, Negrin RS. Revealing lymphoma growth and the efficacy of immune cell therapies using *in vivo* bioluminescence imaging. *Blood* 2003; **101**: 640–648.
30 Baker J, Verneris MR, Ito M, Shizuru JA, Negrin RS. Expansion of cytolytic CD8(+) natural killer T cells with limited capacity for graft-versus-host disease induction due to interferon gamma production. *Blood* 2001; **97**: 2923–2931.
31 Lu PH, Negrin RS. A novel population of expanded human CD3+CD56+ cells derived from T cells with potent *in vivo* antitumor activity in mice with severe combined immunodeficiency. *J Immunol* 1994; **153**: 1687–1696.
32 Leemhuis T, Wells S, Scheffold C, Edinger M, Negrin RS. A phase I trial of autologous cytokine-induced killer cells for the treatment of relapsed Hodgkin disease and non-Hodgkin lymphoma. *Biol Blood Marrow Transplant* 2005; **11**: 181–187.
33 McKee TD, Grandi P, Mok W, Alexandrakis G, Insin N, Zimmer JP *et al*. Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. *Cancer Res* 2006; **66**: 2509–2513.
34 Kim JH, Lee YS, Kim H, Huang JH, Yoon AR, Yun CO. Relaxin expression from tumor-targeting adenoviruses and its intratumoral spread, apoptosis induction, and efficacy. *J Natl Cancer Inst* 2006; **98**: 1482–1493.
35 Breitbach CJ, Paterson JM, Lemay CG, Falls TJ, McGuire A, Parato KA *et al*. Targeted inflammation during oncolytic virus therapy severely compromises tumor blood flow. *Mol Ther* 2007; **15**: 1686–1693.
36 Kirn D, Wang Y, Le Boeuf F, Bell JC, Thorne SH. Targeting of interferon-beta to produce a multi-mechanistic oncolytic vaccinia virus. *PLOS Med* 2007; **4**: e353.
37 Verneris MR, Karami M, Baker J, Jayaswal A, Negrin RS. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8+ T cells. *Blood* 2004; **103**: 3065–3072.
38 Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, *et al*. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 1999; **285**: 727–729.
39 Long EO. Tumor cell recognition by natural killer cells. *Semin Cancer Biol* 2002; **12**: 57–61.
40 Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* 2001; **2**: 255–260.
41 Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003; **3**: 895–902.
42 Clarke MF, Becker MW. Stem cells: the real culprits in cancer? *Sci Am* 2006; **295**: 52–59.
43 Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. *Cell* 2006; **124**: 1111–1115.
44 Li Y, Idamakanti N, Arroyo T, Thorne S, Reid T, Nichols S *et al*. Dual promoter-controlled oncolytic adenovirus CG5757 has strong tumor selectivity and significant antitumor efficacy in preclinical models. *Clin Cancer Res* 2005; **11**: 8845–8855.
45 Banaszynski LA, Chen LC, Maynard-Smith LA, Ooi AG, Wandless TJ. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* 2006; **126**: 995–1004.